THE RESPONSE OF A VESTIGIAL MÜLLERIAN DUCT TO THE FEMALE HORMONE AND THE PERSISTENCE OF SUCH RUDIMENTS IN THE MALE FOWL[1]

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TWO PLATES (EIGHT FIGURES)

INTRODUCTION

In the spring and summer of 1930, the response at progressive developmental stages to the own and opposite sex hormones was studied in Brown Leghorn chicks and young fowls. These experiments have been reported in brief (Juhn and Gustavson, '30), and are described in a separate communication (Juhn, Gustavson, and Gallagher, in press); they were planned to include the reaction of both the primary and the secondary sex characters, and each period of observation therefore terminated with an autopsy record.

During these final dissections a male was discovered showing a much convoluted duct on the left, corresponding in location and extent to the anterior third of the oviduct of the female. The bird had received subcutaneous injections of female hormone for about three weeks and the noticeable degree of development of the müllerian vestige was attributed to a positive response to the hormone. Standards of comparison were sought in persistent female rudiments occurring in other males, both treated and untreated, and to this end sixty-three male fowls of various ages were carefully dissected with particular attention to the regions corresponding

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to the oviduct extension in the hen. Three more cases of persistent müllerian vestiges were discovered; one occurred in young male chick belonging to a series collected for the purpose of establishing data on normal postembryonic development, the other two were found in birds injected with male hormone. Since the presence of the male hormone has been shown to cause no modifications in the female secondary sex characters (Domm, '27, p. 62; Juhn, D'Amour, and Womack, '30), the müllerian rudiments in these three males were all considered adequate for the purpose of comparison with the female-hormone-treated case.

The description of the original oviduct discovered in the female-hormone-injected cockerel together with the additional findings made form the subject of this brief paper; it represents in its biological aspects a part of the program on the biology of sex now continued under the direction of Prof. F. R. Lillie at the Whitman Laboratory of Experimental Zoölogy, The University of Chicago.

The female hormone was prepared by Dr. R. G. Gustavson, of the Department of Phyiological Chemistry, The University of Chicago.

DESCRIPTIVE

1. Oviduct rudiment in a cockerel injected with female hormone

♂ 30-68. Hatched 6/12/30. 8/28/30, feathers plucked from the anterior breast and the saddle. 9/7/30 to 9/23/30, inclusive, daily subcutaneous injections of female hormone, 10 rat units per 25 grams' body weight. (Female hormone 1375, prepared from human pregnancy urine and containing by assay 100 rat units per cubic centimeter.) Body weight and comb length and depth at beginning of experiment: 481 grams, 50 × 24 mm.; at termination of experiment: 520 grams, 52 × 24 mm.

9/27/30. Bird autopsied. The feather sections regenerated during the period of female hormone treatment corresponded exactly to the plumage of the female of the same age.

The cockerel was very much emaciated and showed evidence of tuberculous infection. The dissection showed the presence of the normal male reproductive system, the testes were quite small, as was consistent with the bird's poor general condition, but they showed

no deviation from the normal male juvenile structure on histological examination.

Peripheral and lateral to the left testis was a much convoluted duct. This duct extended in an anterior direction to the septum, in a posterior direction to the second kidney lobe. There was no opening at either duct end and no continuation toward the cloaca. On the right, close examination showed the presence of a small tag of convoluted tissue at the head of the right testis and of a small oval vesicle located just lateral to the wolffian duct and at the level of the posterior margin of the second kidney lobe. There was no tissue continuity between these two müllerian vestiges and no traces of persistent oviducal structure could be located near the cloaca.

There was no difficulty in diagnosing the duct on the left as a vestigial oviduct, for it corresponded in location to the anterior third of the oviduct of the female and also greatly resembled it in gross anatomical structure, though the walls were rather more delicate than in a normal oviduct presenting the same degree of coiling.

The microscopic investigation completely confirmed this first opinion; it was based on cross-sections prepared from different duct levels, all 10 μ thick and stained in iron hematoxylin and Mallory's triple stain.

The sections through the most anterior level of the duct, the region extending diagonally across the kidney toward the septum, showed definite oviduct structure. There was a delicate circular muscle layer, mucous folds, a much folded epithelium—in fact, the typical young oviduct condition.

From a more posterior level, approximately opposite the adrenal, cross-sections showed very similar structure, but a much more pronounced degree of development. The circular muscle layer was very definite and the duct here quite vascular. The mucous folds were somewhat more differentiated than in the anterior region, they extended for some distance into the lumen and were much subdivided, presenting a greater secretory surface. Actual tubular glands were not found, but their primordia were readily determined (fig. 5).

Sections through the region corresponding to the ostium showed a structure closely tallying with the corresponding region in the female. The duct walls were low, the muscle layer delicate, the epithelium arranged in rounded projections and bearing numerous cilia. However, even though the entire region showing this type of structure was sectioned serially, no opening was found (fig. 7).

The sections through the most posterior duct extension again showed an appearance comparable in every way to the albumen-secreting region of the female oviduct.

On the right, the sections through the tag of tissue found near the testis head did not compare to oviducal tissue, rather they resembled the dorsal ligament of the oviduct. The sections through the vesicle were also not strictly comparable to any normal oviduct region, but perhaps might be interpreted as an activation occurring in a much degenerated vestige (fig. 6).

2. Oviduct rudiments in males not treated with female hormone

The three other cases of persistent oviducal vestiges were distributed among two males receiving injections of male hormone, ♂ 30-10, three weeks of age and ♂ 30-78, twenty-one weeks of age at the time of autopsy, respectively, and one normal male chick, ♂ N-S 16, forty-four days of age at dissection. The female vestiges found in these three cases were so similar that one description of their anatomical appearance applies accurately to all. In each instance the oviduct rudiment persisted as a duct on the left side only. Located laterally and peripherally to the left testis, the duct extended somewhat beyond it in a posterior direction; anteriorly, each duct swung diagonally across the kidney to the septum. In each of these three cases the vestigial oviducts were entirely straight tubes with no evidence whatever of coiling. These müllerian rudiments corresponded, as did the first described, to the anterior third of the female oviduct and again no continuation toward the cloaca was demonstrable.

On the right, the female vestiges were represented in each case only by a vesicle distended with fluid, located adjacent to the wolffian duct and approximately at the level of the second kidney lobe. Figure 2 is a photograph of the chick, ♂ N-S 16; it appertains to the Whitman Laboratory collection and therefore no histological material was prepared from the persistent müllerian duct.

Cross-sections were prepared from samples dissected out from different levels of the other two vestigial oviducts as well as the right vesicle.

♂ 30-10. Hatched 6/12/30. Daily subcutaneous injections of male hormone, $\frac{1}{4}$ bird unit per 25 grams' body weight from 6/14/30 to 7/3/30, inclusive. Body weight and comb length at beginning of experiment: 32.0 grams, 11 mm.; at termination of experiment, body weight and comb length and depth: 86.4 grams, 21 × 8 mm. 7/6/30. Chick autopsied; in good condition. The dissections showed an entirely normal male reproductive system and the histological investigation of the testis, the structure usual for the chick of this age. The microscopic appearance of the sections prepared from the anterior and posterior levels of the left müllerian duct proved quite similar,

an entirely characteristic oviduct structure was present, corresponding to the albumen-secreting region of the oviduct of a female chick a few days of age. There was no development of muscle tissue or of differentiation within the mucous folds; these last, in fact, were only beginning to be indicated (fig. 3).

The structure of the walls of the vesicle on the right was not comparable to oviduct tissue from any level, but on the other hand strongly resembled the condition frequently found in the right rudimentary oviduct of the normal female (fig. 8).

♂ 30-78. Hatched 6/12/30. 10/9/30, feathers plucked from the anterior breast and saddle. 10/19/30 to 11/6/30, inclusive, daily subcutaneous injections of male hormone, ½ bird unit per 25 grams' body weight. Body weight and comb length and depth at beginning of experiment: 959 grams, 77 × 53 mm.; at termination of experiment, 1223 grams, 89 × 58 mm. 11/8/30, bird autopsied; cockerel in excellent condition. The feather sections regenerated during the period of male hormone injection were entirely male. The male reproductive system on both gross and microscopic evamination presented the entirely normal appearance for a cockerel of this age.

Cross-sections prepared from the anterior levels of the müllerian duct present on the left showed complete correspondence of structure with that of a normal oviduct from a young female, approximately three weeks of age. There was no development of muscle tissue or of differentiation within the barely indicated mucous folds. Sections through the posterior duct levels, while showing a somewhat further degree of development, gave no indications of functional differentiation. Muscle tissue was absent, but the mucous folds were more clearly set off and extended further into the lumen of the oviduct (fig. 4). The vesicle on the right presented precisely the same lack of structural development described for ♂ 30-10.

DISCUSSION

The right and left müllerian ducts are laid down symmetrically in male embryonic chick of about seventy-two hours' incubation; they persist till about the eighth day, when involution commences, originating at the posterior sections and proceeding anteriorly (Lillie, '19; pp. 194, 402).

Now, it has been shown experimentally by Willier and Yuh ('28) that this normal process of regression to entire disappearance of the female ducts in the male may be suspended by introducing abnormal conditions, such as temperature changes and, possibly, humidity, during the critical periods

of incubation. These authors made their records on the induced modifications of the reproductive system the eighteenth day of incubation, but it seems entirely reasonable to believe that rudiments, persisting beyond the period in development when involution normally occurs, will no longer be exposed to regressive influences and therefore the basis is laid for hermaphrodite conditions in the ducts of the adult.

The extension in length of the müllerian vestiges found may be considered a means of estimating the time of onset of the arrest of the regressive processes. In all the cases listed in this paper as well as in one interesting case of a persisting müllerian vestige in an adult male starling described by Kummerlöwe and Froböse ('30) the oviduct rudiments were represented by the anterior sections only, indicating a comparatively late neutralization of involution.

In the four cases described here, the female vestiges persisted as a duct on the left side only; on the right, they were represented exclusively by small vesicles having no recognizable oviducal structure. In the starling as well, a duct was present on the left side only; here no female vestiges whatever were determined on the right. By far the greater number of cases of persistent müllerian ducts in chick embryos reported by Willier and Yuh also occurred on the left side (p. 94 et seq.). The extremely interesting condition then obtains; regression of the müllerian ducts in the male fowl corresponds in point of symmetry to the behavior of these ducts in the normal female, for, as well known, only the left functional oviduct is present.

The direction of involution is, however, opposite in the two sexes: in the male, incomplete regression is shown by persistence of the anterior section of the müllerian duct, while in the female, the right rudimentary duct corresponds to the posterior levels.

In this connection it may be noted, that Mrs. Dexter, working under the direction of Professor Willier, found that in the normal female chick embryo of sixteen days' incubation the portion of the oviduct superior to the ostium was com-

posed of a solid cord of tissue and this has been confirmed for postembryonic developmental stages by one of us (M. J.). Yet all the oviduct rudiments found in the male fowls described here showed typical oviducal structure with a definite lumen in even their most cranial regions. This may indicate something of an anteroposterior obliteration occurring in the oviduct of the female chick; in fact, the entire question of the involution of these ducts in either sex and particularly in the male with reference to the question of asymmetry is of interest.

The müllerian ducts in the male fowls not treated with female hormone and in the male starling described by Kummerlöwe and Froböse agreed very closely both as to anatomical and microscopic structure. All were straight tubes, showing complete lack of functional development and corresponding in appearance to the oviduct of the very young, immature female chick.

The müllerian duct present in the cockerel receiving injections of female hormone differed strikingly from the preceding in anatomical appearance, for it was a much convoluted, muscular organ, and in microscopic structure, for it presented definite evidence of differentiation in the development of gland primordia, of much subdivided mucous folds, and the particularly well-developed, ciliated epithelium. Finally, the histological investigation showed an agreement of the various duct levels with the corresponding sections of the female oviduct, that is, differentiation into albumen-secreting and ostium regions, and such zones were entirely absent in the other müllerian vestiges. The positive response of the female rudiment in the male fowl to the female sex hormone is thus definitely apparent. It occurs within the limits of the extension of the tissue present at the time of onset of hormone action and there is no question of a determination of organized new growth in a definite direction, such, for example, as a caudal extension toward the cloaca.

These findings seem to indicate rather clearly that the first primordium is laid down, and differentiation up to a certain

point in the müllerian duct occurs, independently of the presence of the female hormone. This conception is further substantiated by the larger size and somewhat further degree of differentiation of the oviducal rudiment in the cockerel 30-78, when compared to the chick 30-10. There is no reason in either case for postulating female hormone presence at any time in development subsequent to hatching; the plumage of the older bird was particularly examined for female modification with negative results, and this examination included preceding plumage stages as well, since the successive juvenile molts do not generally cause replacement of the entire feathering. Yet, both growth, and to some extent differentiation, of the female vestige here had proceeded beyond the stage illustrated in the younger male chick and, certainly, definitely beyond the condition of the oviduct of the just hatched female chick.

The evidence for a similar interpretation of the first stages of development of the oviduct of the female will be considered in another place.

SUMMARY

1. Four cases of persistent oviduct rudiments in male fowls are described.

2. The functional differentiation present in one of these cases due to female hormone administration is compared to the entirely immature condition of the other three untreated oviduct vestiges.

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PLATE 1

EXPLANATION OF FIGURES

1 Photograph of the reproductive system of ♂ 30-68, fifteen weeks of age, showing the persistent müllerian duct vestige peripheral to the left testis. Compare the degree of development and the convolutions present in the duct of this female-hormone-treated cockerel with the müllerian vestige in figure 2, appertaining to an untreated male.

2 Photograph of the reproductive system of ♂ N-S 16, forty-four days of age, illustrating the appearance of the persistent müllerian vestiges in normal and male-hormone-treated cockerels. Note entire absence of convolutions and of muscular development.

3 Detail of cross-section (× 200) of müllerian vestige in ♂ 30-10, treated with male hormone, three weeks of age, showing absence of functional differentiation.

4 Detail of cross-section (× 200) of müllerian vestige in ♂ 30-78, treated with male hormone, twenty-one weeks of age, showing somewhat larger size of duct than in ♂ 30-10, but similar lack of functional differentiation.

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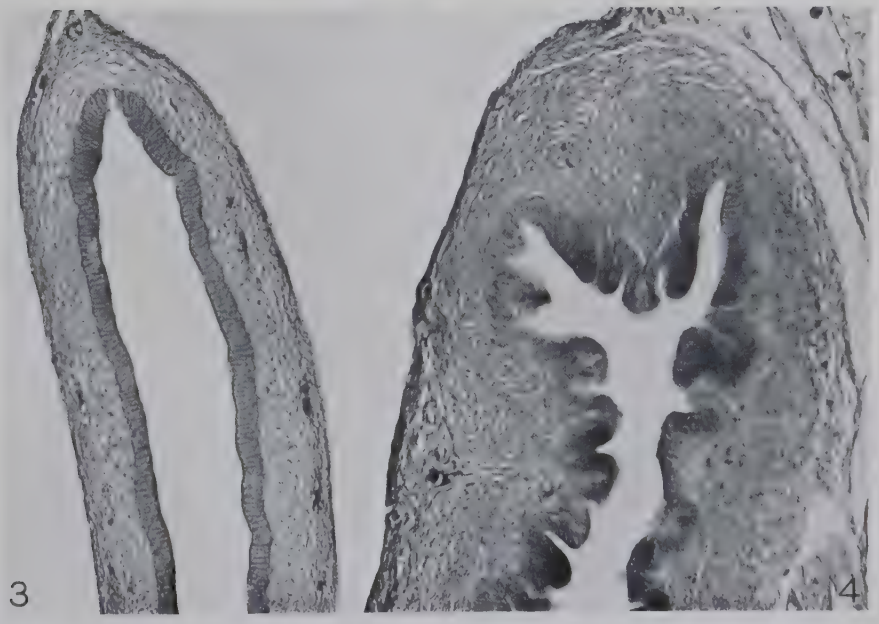
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PLATE 2

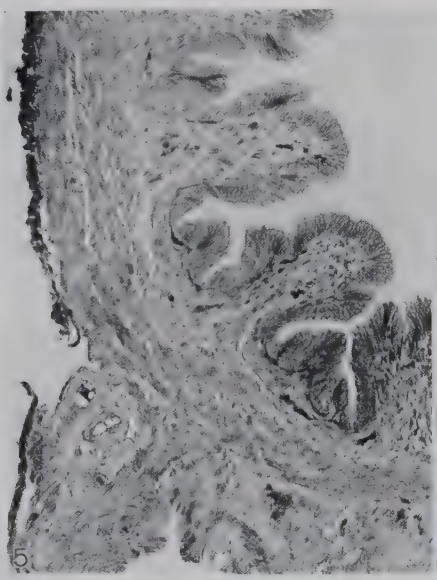
EXPLANATION OF FIGURES

5 Detail of cross-section (× 200) through the region corresponding to the albumen-secreting region of the female oviduct, of the müllerian vestige in ♂ 30-68, treated with female hormone. The pronounced muscular development, primordia of tubular glands, and ciliated epithelium are to be noted in comparison with figures 3 and 4 in plate 1.

6 Detail of cross-section (× 200) through the right vesicle of ♂ 30-68. Compare the indications of activation with the entire lack of differentiation in the corresponding structure in ♂ 30-10.

7 Detail of cross-section (× 200) through the region corresponding to the ostium region of the female oviduct, of the müllerian vestige in ♂ 30-68, treated with female hormone.

8 Detail of cross-section (× 200) through the right vesicle of ♂ 30-10.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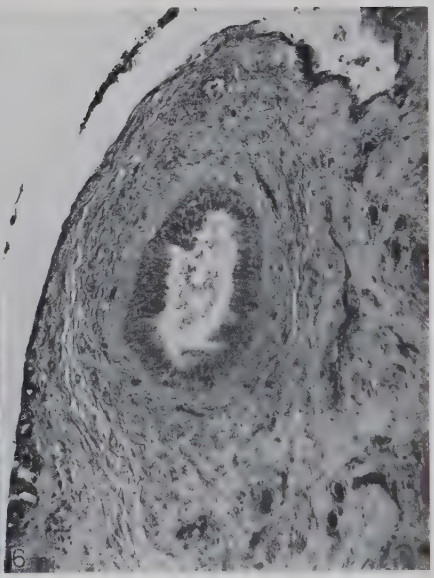
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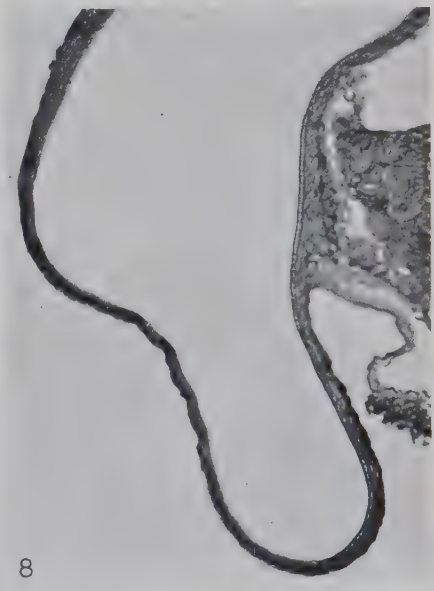
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NOTE ON THE CONTOUR OF THE FORAMEN MAGNUM IN DIFFERENT STRAINS OF THE NORWAY RAT

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ONE FIGURE

While dissecting a series of mature captive gray Norway rats—from the colony of Doctor King—and a series of mature Albinos from The Wistar Institute Colony, my assistant, Miss Meeser, noted that the contour of the foramen magnum was pentagonal in the gray Norways, while in the Albinos it was quadrilateral. This difference in contour can be seen in figure 1, where the skulls in column 2—captive Grays—show the pentagonal contour and those in column 5—Wistar Albinos—the quadrilateral.

The results of a further study of these relations are presented in this note.

In the first instance the contour of the foramen magnum in five different strains of Norway rats was examined. Six skulls were taken for each strain and these were arranged in a vertical column, as shown in figure 1.

MATERIAL USED FOR FIGURE 1

Column 1. Wild gray Norways

These skulls were from full-grown wild rats trapped in or about Philadelphia. The exact ages are, of course, unknown. Though variable, the foramen magnum in this strain has the pentagonal contour. This is brought about by the junction of the two parts of the supra-occipital bone, which form the dorsal border of the foramen, uniting at an angle in the midline.

Column 2. Captive gray Norways from Doctor King's colony

These skulls were from gray Norways kept in captivity from six to nineteen generations (average, fourteen generations). They were all over 600 days of age.

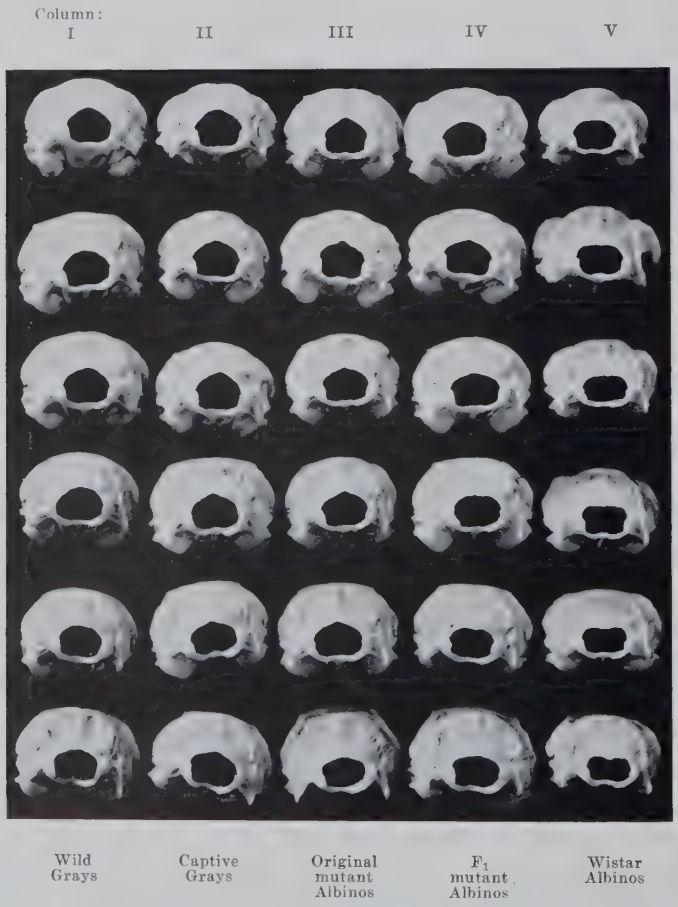

Fig. 1 Showing the form of the contour of the foramen magnum in five strains of Norway rats.

In this strain the pentagonal contour of the foramen is clearly marked, and the skulls in the fourth, fifth, and sixth rows show a slight notch, marking the juncture of the two parts of the supra-occipital bone.

A comparison of the contours shown by the captive Grays —column 2—with those shown by the wild Grays—column 1 —shows that captivity, within the periods given, has not produced any notable alteration in contour.

Column 3. Original mutant Albinos

These skulls were from the original mutant Albinos thrown by the captive Grays in Doctor King's colony.

These mutant Albinos were over 600 days old and were from the fifteenth to seventeenth generations of the captive Grays. The contour of the foramen magnum is in all cases pentagonal, and in several instances a dorsal notch is shown. In general, the contour of the foramen in this strain is like that shown by the captive Grays—their immediate parents. It is concluded from this comparison that Albinism, when it first appears, does not carry with it a change in the contour of the foramen magnum.

Column 4. Mutant Albinos F_1

The skulls here shown are from the F_1 generation from the mutant Albinos. These rats were more than 600 days old. Inspection shows that in some of the skulls the contour is approximately quadrilateral. Thus even at this early period there seems to be a tendency for the contour to be like that found in the Wistar Albinos.

The course of this change must await the examination of skulls from later generations—not at present available. This delayed modification in the contour for mutant Albinos is a matter of special interest, and it seems probable that other responses leading to the characteristic organ composition in the Albinos of our colonies may behave in a like manner.

Column 5. Wistar colony Albinos

These skulls were from Albino rats 800 or more days of age. These rats are from a colony known to have bred in captivity for more than thirty years.

The contour of the foramen magnum is quadrilateral, with at times a more or less clearly marked tooth of bone in the middle of the dorsal boundary. Extreme age is not responsible for the characteristic contour, since it is as well marked in skulls at 450 days of age. The quadrilateral contour thus marks off the Wistar Albinos from the wild Gray, captive Gray, and original mutant strains, while in the F_1 Albino mutant strain the form of the contour is somewhat intermediate.

SIZE OF THE FORAMEN MAGNUM

Inspection of figure 1 suffices to show that the area of the foramen magnum is smallest in the Wistar Albino strain. This is due, mainly at least, to the fact that these animals were smaller than those of the other strains with which they are compared.

At the moment our interest is not in the area of the foramen, but in the relations of the longitudinal and transverse diameters, which will be hereafter designated the length and the breadth, respectively, of the foramen.

These diameters were measured with calipers on the five series of skulls which appear in figure 1, and the ratios of breadth/length are given in table 1.

TABLE 1

The mean of the ratios of breadth/length in the five strains of rats the skulls of which are represented in figure 1

Column	*Strain*	*Breadth/length ratio*
1	Wild Grays	1.32
2	Captive Grays	1.30
3	Original mutant Albinos	1.30
4	Mutant Albinos F_1	1.34
5	Wistar Albinos	1.60

As is seen in table 1, the ratio for the Wistar Albinos is high and well above that for the other strains—a result due to the relatively greater decrease in the value for length.

AGE RELATIONS

The fact that the skulls thus far studied are from mature rats has been emphasized. It remains to determine whether there are age changes in the contour of the foramen magnum

in any of these strains. For this purpose skulls of both young and old rats from the strain of the Wistar Albinos and the strain of captive Grays were used.

For the Wistar Albinos there were examined five skulls of each sex at the respective ages of 30, 60, 90, 150, 250, and 450 days. There is no difference in contour according to sex. At thirty days the foramen is round or oval; at ninety days the quadrilateral contour begins to appear; at 250 days it is pronounced, and at 450 days it occurs in nine out of the ten skulls. In three of these nine skulls the dorsal border of the foramen was convex ventrally. Thus in the Wistar Albinos the quadrilateral contour of the foramen magnum is sometimes found at ninety days—or shortly after puberty—and becomes progressively more marked and more frequent with advancing age.

It is to be noted also that in the Wistar Albino strain the pentagonal contour—characteristic for the gray Norway—is rarely found, and when found, poorly developed.

In the case of the captive gray Norways a similar examination of two series of skulls, at sixty and 600 days of age, respectively, was made. In both age groups the contour of the foramen magnum is pentagonal, so that no significant modification occurs with advancing age in this gray strain.

ON THE CHANGES IN THE VALUES FOR THE LENGTH AND BREADTH OF THE FORAMEN MAGNUM WITH AGE

For the measurements of the length and breadth of the foramen magnum in the Wistar Albino strain five female and five male skulls were used. A group of ten skulls at sixty days and another group of ten skulls at 450 days were measured. These are the same age groups that were used for the study of the form of the contour.

For the captive Gray strain groups of ten skulls at sixty days and ten at 600 days of age were used. Again these were the same groups that had been employed for the determination of the form of the contour.

The length and breadth of the foramen magnum were measured in each skull and the ratio of breadth/length determined.

The averages of these values were obtained for each group in the two strains, and these are presented in table 2.

The data in table 2 show that both diameters of the foramen magnum are larger in the captive Grays than in the Wistar Albinos. This was to be expected, since the captive Grays are larger animals.

They also show that in both strains the breadth increases with age, while the length decreases. In both strains the

TABLE 2

Giving the average values for the breadth and length of the foramen magnum and the ratio of the breadth/length in the two age groups of the Wistar Albinos and the captive Grays

AGE, DAYS	BREADTH, MM.	LENGTH, MM.	RATIO, $\frac{B}{L}$
Wistar Albinos:			
(10) 60	6.38	4.97	1.28
(10) 450	6.49	4.03	1.61[1]
	(101.7 per cent)	(81.1 per cent)	
Captive Grays:			
(10) 60	6.60	5.31	1.24
(10) 600	6.76	5.24	1.29[1]
	(102.4 per cent)	(98.7 per cent)	

[1] It may be noted that the ratio for the Wistar Albinos at 450 days in table 2 is 1.61, while the ratio for a group of the same strain over 800 days of age, as given in table 1, is 1.60. Similarly, the group of captive Grays measured for table 2 gives a ratio of 1.29, while a different group measured for table 1 gives 1.30. The similarity of the ratios for different groups within the same strain indicates constancy in the relations.

increase in breadth is slight (Wistar Albinos, + 1.7 per cent; captive Grays, + 2.4 per cent).

On the other hand, there is a large reduction in length in the case of the Wistar Albinos, while in the case of the captive Grays this reduction is slight.

In both strains this reduction is due to a downward growth of the edge of the supra-occipital bone. In the Wistar Albinos this downward growth is very considerable, thus giving a straight dorsal border for the foramen or even a ventrally extended lip or tooth in the midline.

COMPARISON WITH THE FORAMEN MAGNUM IN MAN

A comparison of this change of shape with age in the rat has been made with that occurring in man. It is sufficient to state that since in man the longer axis of the foramen is longitudinal, while in the rat it is transverse, the values for the corresponding ratios obtained are quite different. Yet the human data serve to show a like change in the relations of the two diameters with advancing age.

The measurements for man were made on two series of skulls. For the first series I am indebted to the courtesy of Dr. J. Parsons Schaeffer, Professor of Anatomy at Jefferson

TABLE 3

Average breadth and length of the foramen magnum in human skulls at different ages

	AGE	BREADTH, MM.	LENGTH, MM.	RATIO, $\frac{B}{L}$
Series 1:				
Young group (7)	1 year	19.1	24.1	0.79
Mature group (3)	(?)	30.9	35.5	0.87
		(161.7)	(147.3)	
Series 2:				
Young group (5)	1 year 2 months	20.6	27.1	0.76
Mature group (5)	(?)	29.8	35.3	0.84
		(144.7)	(130.2)	

Medical College, Philadelphia. The other measurements were based on a series of skulls in the collection of The Wistar Institute.

In the first series the ages of the young group, seven cases, ranged from an eight-months fetus to a three-year child, giving an average age of one year, and for the mature group, three cases, the ages were unknown.

In the second series the ages of the young group, five cases, ranged from birth to three years, giving an average age of one year two months, and in this instance also the ages for the mature group, five cases, were not known. The data are given in table 3.

The data in table 3 show that in both series the values for the breadth and for the length are greater in the mature group. As indicated by the percentage relations of the

values for the mature groups, the relative increase in breadth is greater than that in length, and as a consequence the ratio for the mature group is higher than that for the young group. This indicates the same change in the relations of the two diameters as that shown by the data for the rat in table 2.

We conclude, therefore, that in both man and the rat the changes in the relations of the two diameters with advancing age are similar.

CONCLUSIONS

1. The contour of the foramen magnum in the mature Wistar Albinos differs from that in the mature gray Norways, being quadrilateral in the Wistar Albinos and pentagonal in the gray Norways.

2. The quadrilateral contour begins to appear in the Wistar Albinos at about ninety days and becomes more pronounced with advancing age.

3. The quadrilateral contour is brought about by the extension ventrad of the rim of the supra-occipital bone.

4. With advancing age the length of the foramen magnum decreases. This decrease is most marked in the Wistar Albinos.

5. The contour of the foramen magnum in the captive Grays is similar to that found in the wild Grays, despite the fact that the captive Grays have been in captivity for an average of fourteen generations.

6. The original albino Mutants derived from the captive Grays show the same pentagonal contour as do their parents. Thus the quadrilateral contour does not appear in the original mutant Albinos.

7. The F_1 generation from the original mutant Albinos shows a slight change in the contour of the foramen magnum, which tends toward the contour found in the Wistar Albinos. The examination of later generations of this strain must be made before this apparent modification can be discussed.

8. With advancing age the relative values of the two diameters of the foramen magnum undergo the same changes in man as in the rat.

THE RELATION OF THE SCIATIC NERVE TO THE PIRIFORMIS MUSCLE IN AMERICAN WHITES AND NEGROES[1]

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In the majority of cases the relation of the sciatic nerve to the piriformis muscle is a deep one, the nerve lying ventral to the muscle and emerging from its inferior border to continue distalward. Variations from this relationship are chiefly the partial or complete division of the muscle by a part or the whole of the nerve. These variations have been noted by many anatomists and the incidence is usually found to be between 10 per cent and 20 per cent; e.g., Eisler, 18 per cent; Paterson, 13 per cent; LeDouble, 17 per cent; Bardeen and Elting, 10 per cent. These reports have all been based on whites except for that by Bardeen and Elting. Their observations from the Anatomical Laboratory of Johns Hopkins University were made on dissecting-room material composed of both white and negro bodies—material quite similar to that of this laboratory. They found that "neither sex, race, side of body nor skeletal conditions seemed to have intimate association with the natural separation of the sciatic nerve."

The dissecting-room material in this laboratory affords an opportunity to compare further the incidence of variation of the relations of these structures in whites and negroes. Both the nerve and muscle are large enough to eliminate the possibility of destruction during routine dissection. With the cooperation of the students, records of this relationship in

[1] Aid in helping to carry out this research was received from the Rockefeller Research Fund.

the cadavers have been kept for the past six years. A summary of the incidence of variations in the relative positions of the nerve and muscle is given in the table; differences between race, sex, and side of the body are included.

The incidence of bilateral and unilateral variation is approximately the same. However, unilateral variation occurs

TABLE 1

Variation from the usual relationship between the sciatic nerve and the piriformis muscle in cadavers with reference to race, sex, and side of body

RACE	SEX	BODIES			SIDES		
		Number	Incidence	Per cent	Number	Incidence	Per cent
White	Male	102	Both sides only, 11 Right only, 5 Left only, 6 Total bodies, 22	22	204	Both sides only, 22 Right only, 5 Left only, 6 Total sides, 33	16
Negro	Male	81	Both sides only, 6 Right only, 3 Left only, 5 Total bodies, 14	17	162	Both sides only, 12 Right only, 3 Left only, 5 Total sides, 20	12
White	Female	15	Both sides only, 1 Right only, 0 Left only, 0 Total bodies, 1	7	30	Both sides only, 2 Right only, 0 Left only, 0 Total sides, 2	7
Negro	Female	34	Both sides only, 3 Right only, 1 Left only, 2 Total bodies, 6	18	68	Both sides only, 6 Right only, 1 Left only, 2 Total sides, 9	13
Total		232	Both sides only, 21 Right only, 9 Left only, 13 Total bodies, 43	19	464	Both sides only, 42 Right only, 9 Left only, 13 Total sides, 64	14

slightly more often on the left side than on the right in all the groups except the white female group, which is too small for statistical comparisons. The number in this group is inadequate also for a racial comparison of females. A comparison between whites and negroes in the male group shows the whites to be somewhat more variable than the negroes. This is interesting because of the assertion of Rosenmüller,

questioned by both Testut and LeDouble, that a premature bifurcation of the sciatic nerve (which almost invariably is the case in an altered relationship of the nerve and the piriformis muscle) occurs more often in northern races than in meridional (southern) races.

The findings on this material (excluding the white female group which is too small for comparison) may be summarized as follows: the whites are more variable than the negroes; no sex difference is present in the negroes; and the left side is very slightly, but consistently, more variable than the right.

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CONGENITAL ABSENCE OF THE KIDNEY. AN INTERPRETATION BASED ON A 10-MM. HUMAN EMBRYO EXHIBITING UNILATERAL RENAL AGENESIS

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FIVE TEXT FIGURES AND FIVE PLATES (EIGHTEEN FIGURES)

As various authors have pointed out, unilateral absence of the kidney has been known since the time of Aristotle, who observed that although an animal is never found without a heart yet, indeed, it may be found "without spleen, also with two spleens and with one kidney." In man, according to the most recent survey of the literature (Collins, '31), the incidence of cases is less than 1:1000, in which respect it resembles the horseshoe kidney; also it seems to occur more often on the left than on the right side and somewhat more frequently in men than in women. But, as Braasch has pointed out in his discussion of Collins' paper, it is impossible to rule out early destruction of one kidney with disappearance of most of the renal and ureteral tissue, so that all cases in which the kidney is lacking cannot be attributed to embryonic renal agenesis. Nor is it always possible, because of resorption of embryonic vestiges, to reconstruct the exact sequence of events. Hence, the discovery of a young human embryo in which the kidney of one side is in process of disappearance provides, for the first time, direct information about the cause of this malformation in man.

The embryo in question, collected by C. M. Jackson in 1905 (no. 134 of the Minnesota Collection), measured 10 mm., crown-rump, in alcohol. Although it was slightly macerated,

having been deposited for a year in weak formalin by the attending physician, all its tissues were easily recognizable and suitable for reconstruction. Aside from the nephric system the only gross defect appeared in the spinal cord, the lumen of which, in the region below the heart, was cross-shaped when viewed in transverse section. In addition, the ventral pancreas had not yet appeared and the dorsal pancreas was less developed than it should have been at this stage.

As revealed by graphic reconstruction (pl. 1) the right Wolffian duct has reached the cloaca and has given rise to a ureter which, in turn, has grown into the blastema of that side and is apparently developing into a normal kidney. On the left side (*L.W.d.*) the Wolffian duct, in its downgrowth from the pronephros, has reached no farther than the level of the vitelline artery. Furthermore, it has a larger diameter than the one on the right side (fig. 10), suggesting that it has been dilated by the excretions of mesonephric tubules to which it is attached. If so, this is the first physiological evidence of function in the human mesonephros.[1]

Following the failure of the left Wolffian duct to reach the cloaca, no ureter has developed on that side; nevertheless, the left blastema has appeared (figs. 6, 7, and 12), although it is not differentiating into tubules. The significance of this independent origin of the blastema is discussed in connection with experimental findings (p. 332), but it should be noted at this time that the inductive influence of the right ureter is not sufficient to activate the nephrogenic tissue of the left side even though the two blastemas are fused. This is in accord with Brown's observations on a strain of mouse embryos with inherited kidney defects. She states that it is

[1] In chick embryos, it has been shown that mechanical distention of the allantois by mesonephric excretion is an essential factor in the development of that organ, for, with total absence of distending fluid, it remains an abortive organ (Boyden, '24). In the human embryo with its much less developed mesonephros, there is a correspondingly less well-developed allantoic duct. Quite likely, however, in the light of the evidence presented here, there is sufficient excretion to act as a mechanical factor in the early development of the urinary bladder.

necessary for the ureter to enter the blastema before the latter will differentiate into tubules.

Further examination of plate 1 reveals an incomplete development of the Wolffian bodies. In embryos of this length (between 8 and 12 mm.) there should be from thirty-three to thirty-eight mesonephric tubules (Felix; Shikinami). Yet in this embryo there are only twelve mesonephric corpuscles in the left Wolffian body and seventeen in the right. We are thus dealing with an arrest in development of the mesonephroi—presumably the factor underlying renal agenesis.

Apparently, also, this retardation in growth is manifested by the lower position of the kidney anlages which are just entering the umbilical crotch (compare fig. 4, McClure and Butler, illustrating a 11-mm. embryo in which the kidneys are more than halfway through the umbilical crotch).[2]

Although, normally, the umbilical arteries merely tend to bring the kidneys together as they migrate upward into the lumbar region, the two renal blastemas of this specimen (figs. 7 and 12) are so closely hemmed in by the surrounding arteries and veins that they cannot escape fusion. In addition to the general narrowing it should be noted that the left umbilical artery branches from the aorta at a more acute angle than the other, and that the left posterior cardinal vein (from the undeveloped portion of the Wolffian body down) is not only larger than usual, but larger than the corresponding vein on the right side (fig. 1).

Here, then, for the first time, may be viewed the critical stage in the formation of a horseshoe kidney. Besides confirming the theory of Lewis and Papez regarding the rôle played by the umbilical vessels in obstructing the ascent of

[2] McClure and Butler explain the presence of the abrupt bend in the posterior cardinal veins as having been brought about by the migration into this region of the permanent kidneys. But in embryo 134 (pl. 1) it may be noted that the bend occurs in the absence of a normal kidney on the left side and before either kidney has reached the bend. It seems more probable, therefore, that this acute bend should be attributed to the increasing pressure of the umbilical arteries and the placental circulation (compare figs. 1 and 7).

the kidneys (Boyden, '31), this potential case of horseshoe kidney offers alternative suggestions regarding the factors

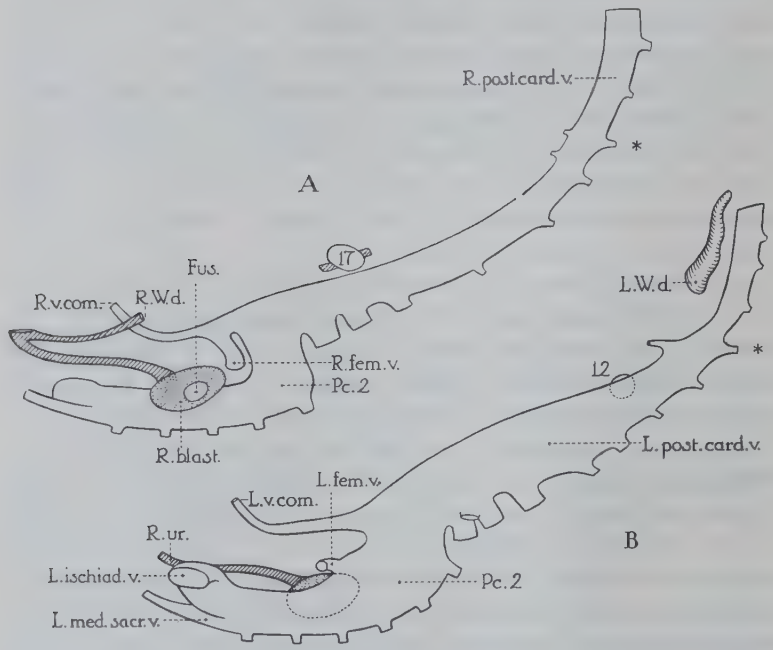

Fig. 1 Graphic reconstruction of right (*A*) and left (*B*) posterior cardinal veins of a 10-mm. human embryo (no. 134 of Minnesota Collection). × 33. Drawn from left side, to illustrate greater development of left veins (compare pls. 1 and 2 for orientation). *Fus.*, area of fusion between left and right blastemas; *L.* and *R.v.com.*, vena comitans of left and right umbilical arteries (each vein courses ventrolaterally to its respective umbilical artery until it reaches the caudal umbilical wall. There it anastomoses with the vein of the other side and with the umbilical veins. These may be the veins which McClure and Butler designate in later stages as the "caudal mesonephric branches of the posterior cardinal veins"); *Pc.2*, second portion of posterior cardinal vein; *asterisk*, designating level of vitelline artery; *12* and *17*, lowest glomeruli of left and right Wolffian bodies, respectively (compare pl. 1).

which underlie this narrowing of the pelvic isthmus. Either there is a lack of expansion of the caudal portion of the body cavity, following lack of development of the caudal portion of the Wolffian bodies or general retardation of caudal

growth; or else the retarding of the upward migration of the kidney has permitted the surrounding vessels to encroach on the space normally occupied by the kidneys before the latter reach their destination. In either case, arrested development of the Wolffian bodies would seem to be the primary cause of both anomalies in this embryo—namely, unilateral renal agenesis and fusion of the renal anlages.[3]

DESCRIPTION OF AN ADULT CASE

Of special interest, in relation to the 10-mm. embryo just described, is a dissecting-room case of absence of the left kidney in a man seventy-two years of age. The patient had been a common laborer, always in good health until the attack of bronchopneumonia that resulted in his death. The genito-urinary history was entirely negative.

In dissecting this subject no vestige of the left kidney could be found and the left suprarenal gland was disc-shaped, indicating that it had never been molded by a kidney. On the right side, kidney and suprarenal gland were normal in every respect, as was also the bladder. But on the left side the ductus deferens, seminal vesicle, and ureter were absent (figs. 8 and 9). Gross examination of the left testis showed a normal organ with ductus deferens extending only as far as the subcutaneous ring. Since the seminal vesicle and adjacent portion of the ductus deferens are derived from the distal end of the Wolffian duct, there can be no doubt but that congenital absence of the left kidney in this case was due to defective development of the distal portion of the left Wolffian body.

To what extent such extensive arrest in development occurs cannot be estimated from a study of adult cases. For, as Collins has pointed out in a personal communication, the literature is very unreliable in its correlation of absence of

[3] This explanation should not be confused with the erroneous theory of arrested development of the kidney proposed by Meckel in 1812. According to Schlesinger, Meckel believed that fusion of right and left blastemas represented a preliminary stage in development that was normally followed by separation of the two kidneys; and that arrest in development caused merely a persistence of this early stage of fusion.

the ductus deferens and seminal vesicle with absence of the kidney; and, in females, absence of the ureter does not give much information regarding the extent of mesonephric deficiency. Nevertheless it is significant that Collins' data include forty-four cases of unilateral absence of ductus deferens and seminal vesicle on the same side as the missing kidney.

Regarding the ureter, Collins finds that in 581 cases of unilateral absence of the kidney, the urinary duct of the defective side was missing in 413 cases (71.07 per cent) and definitely reported as present in only forty-two cases (7.2 per cent); that is, it was absent in approximately 90 per cent of the cases in which the ureter was mentioned. Whether or not the remaining 126 cases (21.73 per cent) would cancel each other as a balanced error is debatable. Certainly, it is clear that all of the 581 cannot be cases of embryonic renal agenesis, but represent absorption of the kidney after it was formed. For in at least forty-nine instances (there is also mention of renal vessels in ninety-four other cases) renal veins were noted on the defective side; and we know from the work of McClure and Butler that renal veins do not develop until the kidneys have formed and have reached the position which they will occupy in the adult (22-mm. stage). In the great majority of cases, however, it would seem certain that congenital absence of the kidney is associated with congenital absence of the ureter.

AGENESIS OF THE URETER FOLLOWING EXPERIMENTAL OBSTRUCTION OF THE WOLFFIAN DUCTS

Turning now to the blastema, let us consider evidence of its persistence in the absence of the ureter. In the following experiments it will be shown that the nephrogenic component of the kidney (the blastema) does not differentiate into tubules in the absence of the ureteric component. Nevertheless, the former always appears as an anlage, in spite of lesions that prevent the formation of the ureter. Here, then, is striking evidence that failure of the kidney to develop is

not due to failure of appearance of its nephrogenic component.

The experiments here outlined represent a continuation of those reported in 1924 and briefly mentioned in 1927—experiments in which the Wolffian ducts of young chick embryos of approximately thirty-six to fifty-six hours of incubation were

TABLE 1

Experimental agenesis of the ureter

CHICK NO.	LEVEL OF LESION	AGE AT OPERATION		AGE AT FIXATION		URETERS		BLASTEMAS	
a. Both Wolffian ducts interrupted terminally									
	Somite	*Somites*	*Hours*	*Days*	*+ Hours*	*L.*	*R.*	*L.*	*R.*
215	19th	21	39	5	13	—	—	+	+
216	24th	24	39	5	16	—	—	+	+
231	25th	22	40	4	14	—	—	+	+
190	29th	25	50	6	0	—	—	+	+
b. Both ducts operated upon, terminally; one interrupted									
239	10th	10	40	4	21	+	—	+	+
252	12th	12	35	4	23	+	—	+	+
212	19th	19	38	3	12	—	+	+	+
29	29th	28	51	3	21	+	—	+	+
28	30th	27	51	5	18	—	+	+	+
c. One duct operated upon, terminally									
163	30th	28	49	5	10	—	+	+	+
166	30th	30	50	6	9	+	—	+	+
174	30th	30	52	6	1	+	—	+	+
d. Both ducts operated upon subterminally; distal end of one reaches cloaca									
59	26th	27	52	5	20	—	—[1]	+	+
e. Both ducts operated upon subterminally; both distal ends reach cloaca									
101	26th	32	56	5	8	—[2]	+	+	+
21	27th	29	49	5	9	—[1]	+	+	+

[1] Stump of Wolffian duct reaches cloaca, but is too short to form ureter.

[2] Long segment of Wolffian duct reaches cloaca, but no ureter is formed.

injured, either at their growing points or behind the zone of differentiation; following which the eggs were reincubated for one to five days. From several hundred experiments numerous embryos were sectioned for study. Out of twenty-nine that were old enough to contain the blastema, half of them showed that the Wolffian ducts of one or both sides had been successfully interrupted, with consequent failure in development of the ureteric bud (table 1). Yet in spite of absence

of one or both ureters, and of the fact that in some cases the lesion was severe enough to sever the spinal cord (e.g., figs. 4 and 22) or to split the wing buds (e.g., figs. 21 and 3) or the leg buds (figs. 23, 19, and 5), nevertheless both blastemas appeared.

From a study of these embryos it therefore becomes apparent that differentiation of metanephric tubules is not a direct continuation of that repetitive process which gives rise to mesonephric tubules, even though the nephrogenic tissue of the two organs is at first continued (Sedgwick, '80). For, whenever the nephrotome is interrupted experimentally at any level in the region of the future Wolffian body, the Wolffian duct and tubules fail to appear caudad to the lesion. Yet the metanephric blastema invariably appears, even when the lesion is as low as the thirtieth somite (table 1).[4]

Another characteristic of the metanephric blastema is its apparent power to assume the configuration of the embryonic

[4] In the experiments of Willier and Rawles and of Hunt, which consisted of transplanting portions of the blastoderm of chick embryos (under the age of twelve somites) to the chorio-allantoic membrane, it is shown that the older the blastoderm (i.e., the more caudad Hensen's node is at the time of transplantation) the more likely it is that the mesonephros will develop. Willier and Rawles interpret these interesting findings to mean not only that the segregation of the mesonephros is a repetitive process—each unit of tubules arising in serial order as a segregate—but also that some relation exists between the number of units segregated and their capacity to survive in a graft. Yet in the writer's experiments mesonephric tubules fail to appear in the absence of the Wolffian duct and no trace of nephrogenic tissue of the Wolffian body is visible (figs. 13 and 16) except in the earliest stages (fig. 1, Boyden, '27). Hunt also notes that in grafts of Hensen's node mesonephric tubules are found attached to the Wolffian duct. If this dependence of tubule formation upon the duct is substantiated, it would seem necessary to add another step to the interesting interpretation of Willier and Rawles regarding the organization of the mesonephros—namely, to postulate that the rôle of Hensen's node is to organize the pronephros, the parent tissue of the Wolffian duct. The latter, as it moves caudad, will then induce serial formation of mesonephric tubules. As evidence of the dependence of the mesonephros upon the formation of the pronephros, it should be recalled that in normal development the Wolffian duct appears in advance of the mesonephric tubule. Secondly, attention is called to embryos 239 and 252 (table 1) in which unilateral interruption of the nephrotome in the region just above the mesonephros has resulted in lack of formation of any mesonephric ducts or tubules on that side, caudad to the lesion.

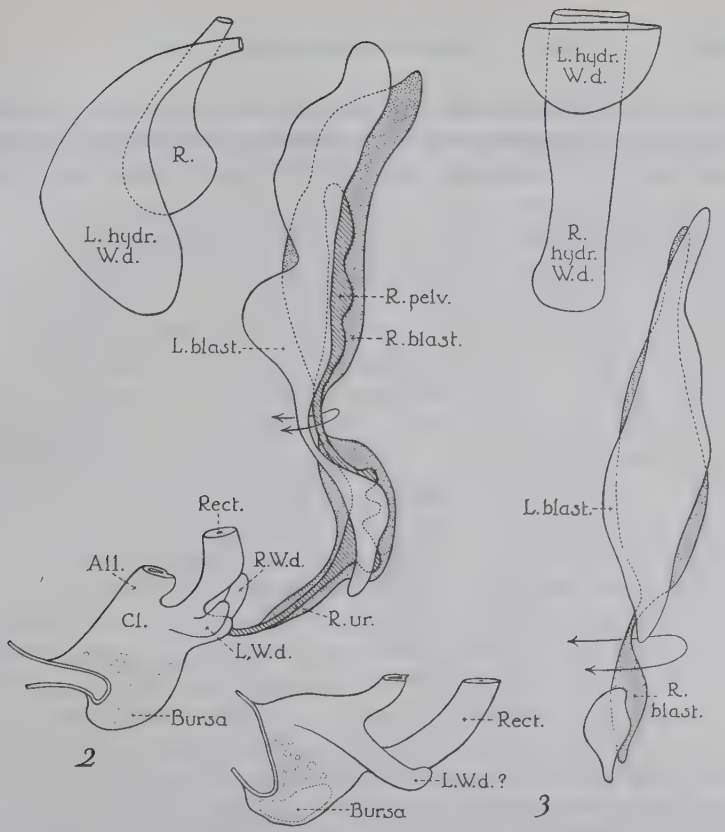

Fig. 2 Reconstruction of chick embryo no. 21 (five days nine hours). × 37.5. Both Wolffian ducts interrupted subterminally at level of twenty-seventh somite, during twenty-nine-somite stage (forty-nine hours of incubation). Lower ends of each duct (*L* and *R.W.d.*) reached cloaca (*Cl.*). Upper ends are hydronephrotic (*L.* and *R.hydr.W.d.*). Right Wolffian duct (*R.W.d.*) gives rise to ureter (*R.ur.*). Left Wolffian duct is probably too short to form ureter (compare position of ureteric bud in early stages: fig. 14, Boyden, '22). Left blastema (*L.bl.*) assumes same shape as right, notwithstanding absence of left ureter. Left blastema is compressed by umbilical arteries (curved arrow). *All.*, allantois; *Bursa*, bursa of Fabricius; *Rect.*, rectum. (For sections through blastemas at level of umbilical arteries, see fig. 14.)

Fig. 3 Graphic reconstruction of chick embryo no. 215 (five days thirteen hours. × 37.5. Wolffian ducts interrupted subterminally at level of nineteenth somite, during twenty-first somite stage (thirty-nine hours of incubation). Wing buds cleft by lesion (compare fig. 21). Ends of Wolffian ducts reach cloaca (?), but are too short to form ureteric buds. Nevertheless each blastema appears (*L* and *R.blast.*) in its usual place. At level of umbilical crotch (curved arrow) the left blastema is obliterated, being compressed between the umbilical arteries. (For section through blastemas above level of arrow, see fig. 16.)

kidney, notwithstanding the absence of the ureter. When one studies the reconstruction of such an embryo as is shown in figure 2, it becomes clear that the reason why the kidney

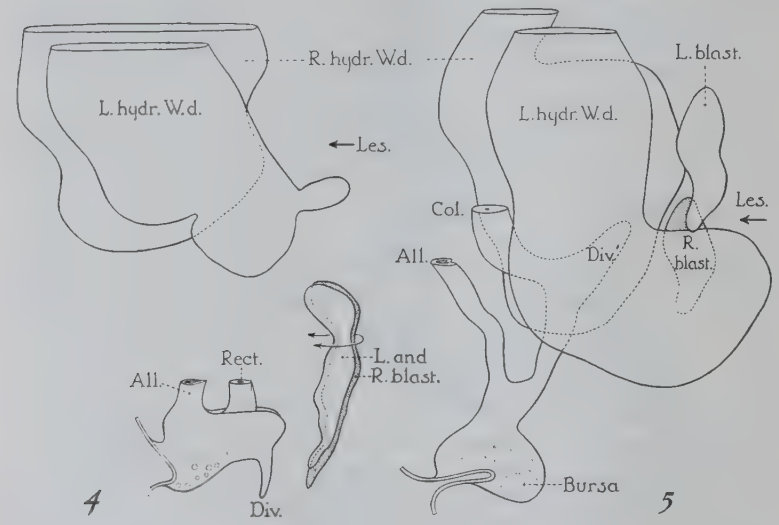

Fig. 4 Graphic reconstruction of chick embryo no. 231 (four days fourteen hours). × 30. Both Wolffian ducts interrupted terminally at level of future twenty-fifth somite, during twenty-two-somite stage (forty hours of incubation). No trace of Wolffian ducts on cloaca. Lesion was severe enough to sever spinal cord and dislocate the two halves (see fig. 22). Nevertheless, both blastemas appeared in absence of ureteric buds. Note anomalous caudal diverticulum of cloaca (*Div.*). *All.*, allantois; *Les.*, level of lesion; *Rect.*, rectum; *curved arrows*, region where umbilical arteries compress right and left blastema (see fig. 17).

Fig. 5 Graphic reconstruction of chick embryo no. 190 (six days). × 30. Both Wolffian ducts interrupted terminally, at level of future twenty-ninth somite, during twenty-five-somite stage (fifty hours of incubation). Right leg bud cleft by lesion (see fig. 19). Lesion sufficiently severe to intercept aorta and change direction of colon (see anomalous diverticulum, *Div.*). Note enormous distention of Wolffian ducts (*L.* and *R.hyd.W.d.*) which greatly restricts available space for development of blastema, but does not prevent its appearance.

is devoid of tubules at the level of the umbilical crotch is not because the pelvis of the kidney is narrower there, but because there is hardly any blastema at that level. To be sure, the underlying cause of this configuration may well be the pres-

sure exerted upon the blastema by the umbilical arteries, which seems to increase with age (compare figs. 4 and 3; 17 and 14). In figure 3, for instance, the isthmus of the left blastema is entirely obliterated by the arteries. In still other places it is prevented from developing in its usual position on the median side of the posterior cardinal vein by the pressure of one or both of the enormously distended hydronephrotic Wolffian ducts (figs. 4 and 18).[5]

The ultimate fate of the blastema that forms in the absence of a ureteric bud is not known with certainty. In the oldest embryo of the series (no. 166: six days nine hours; fig. 2, Boyden, '27), it is still visible. But in view of its lessening density one may assume that it eventually disappears. This is rendered the more probable by the fact that in the 10-mm. human embryo described above, the left blastema was not differentiating even though it was in contact with the normal blastema of the other side. Secondly, Nicholson has made the observation that "in not a single case is there a record of a solid or, as we should expect if the blastema were self differentiated, cystic mass or isolated cyst at or near the level of the bifurcation of the aorta."

It would seem therefore that while the blastema does not persist in the absence of the ureter, sufficient evidence has never been advanced to warrant the theory that congenital absence of the kidney is due primarily to lack of appearance of the blastema.

DISCUSSION

There remains a third possible explanation of embryonic renal agenesis—namely, lack of union of blastema and ureteric bud. Such an occurrence has been clearly demon-

[5] In hydronephrosis of the Wolffian body, it is of interest that the part of the mesonephric tubule which becomes most dilated is the glomerulus (arrow, fig. 18); whereas in hydronephrosis of the adult human kidney, only the collecting ducts dilate—the glomeruli and tubules becoming atrophic. Probably this is due to the fact that the mesonephros is not surrounded by an unyielding capsule so that the blood supply of the tubule is not interrupted and there is still room for dilatation of the glomeruli.

strated by Brown ('31), who has made a careful embryological study of a strain of brown mice (descended from a group of animals treated with comparatively light doses of unfiltered x-rays) in which the incidence of inherited eye, leg, and kidney defects had been increased by selection. Embryonic material was obtained by caesarian operations, so timed as to provide a graded series of embryos through the critical period of kidney formation.

Analysis of this extensive series shows retardation in development of the ureteric bud, in consequence of which it was unable to overcome the mechanical obstacles that prevented its union with the blastema. Brown classifies the defective ureters into six groups ranging from total absence of the bud to ducts that were in contact with the blastema. In thirteen- to fourteen-day embryos of the last group the ureter was so late in reaching its destination that tunics had begun to form around it and this may offer partial explanation for the fact that in some cases contact of the two anlages had induced only rudimentary, if any, differentiation of tubules. In other cases in which the ureter was far removed from the blastema the latter was in process of disappearance. But no case is recorded in which the blastema had failed to appear. In addition there were rudimentary kidneys in which retardation of development continued after union of renal anlages.

This interesting series described by Brown differs from the human and chick embryos described above,[6] in that retardation of development operates directly upon the ureter instead of through its parent tissue, the Wolffian duct. Undoubtedly this offers an explanation for most human cases in which vestiges of the ureter remain on the side of the missing kidney.

Brown also describes constriction of the distal end of the Wolffian duct from the cloaca, after the ureteric bud had developed, also constriction of the ureter itself at the point

[6] With the exception of embryo 101, the only case in which the ureteric bud failed to develop after a sizable portion of the Wolffian duct had reached the cloaca (table 1).

where it joins the cloaca. This defect recalls the recent important contribution of Chwalla, who in an elaborate restudy of the development of the human cloaca has demonstrated the existence of a septum that closes the end of the ureter at the very moment that it acquires a separate opening from the Wolffian duct. Normally, this septum persists from about the 12- to the 24-mm. stage, but has been found in a 79-mm. fetus. This septum undoubtedly explains an obscure point in human pathology, namely, the occurrence of congenital dilatation of the ureter.

Again, one may ask why retardation in development of these mouse embryos has not resulted in fusion of the kidneys, as noted in a previous article (Boyden, '31)? Apparently this is due to the marked twisting of the body to the right which would tend to keep the kidneys apart. Brown considers that the mechanical obstruction resulting from this twisting of the body is a contributing factor in preventing the defective ureteric bud from reaching the blastema.

Finally, existence of this defective strain of mice raises the question as to whether or not kidney defects in man may not have a genetic basis, especially since we now have proof, at least in one human case, that unilateral renal agenesis can be attributed to arrest in development of the Wolffian body.

CONCLUSIONS

A 10-mm. human embryo is described which exhibits the critical stage in the production of two congenital anomalies of the kidney—unilateral renal agenesis and horseshoe kidney. The underlying defect seems to be an arrest in development of the caudal portion of the mesonephros, as a result of which the left Wolffian duct fails to reach the cloaca and therefore to give rise to a ureteric bud. In its absence the left blastema fuses with that of the right to form a potential horseshoe kidney.

A review of adult cases of congenital absence of the kidney —including a new case with associated absence of the ductus deferens and seminal vesicle—demonstrates that more than

three-fourths of the cases of renal agenesis are characterized by the absence of the ureter.

Ureteral agenesis may be produced experimentally in chick embryos by interrupting the Wolffian duct in its zone of differentiation. Despite the absence of the ureter, the blastema appears and assumes the form of the embryonic kidney, but fails to differentiate into tubules.

An analysis of all data leads to the conclusion that no evidence has yet been presented which justifies the theory that absence of the kidney is due to lack of appearance of the metanephric blastema; on the contrary, it appears to be due to retardation in development of the ureter, to embryonic agenesis of the ureter, or to arrest in development of the parent tissue of the ureter.

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PLATE 1

EXPLANATION OF FIGURE

6 Graphic reconstruction of 10-mm. human embryo (no. 134 of Minnesota Collection) illustrating arrested development of Wolffian bodies, failure of left Wolffian duct to reach cloaca, and fusion of left blastema with right kidney as latter is entering umbilical crotch. (For relations of veins, see figs. 1 and 7; for cross-sections of embryo, see figs. 10 to 12). × 55.

ABBREVIATIONS

All., allantois
Ao., aorta
Caec., caecum
Cl.memb., cloacal membrane
C.b.d., common bile duct
D.pan., dorsal pancreas
Inf.mes.a., inferior mesenteric artery
Isch.br. of r.umb.a., right ischiatic artery
L.umb.a., left umbilical artery
L.W.d., hydronephrotic left Wolffian duct
Rect., rectum
R. and *L.blast.*, right and left renal blastema
R.ur., right ureter
R.W.d., right Wolffian duct
Stom., stomach
Vit.a., vitelline artery
12, lowest glomerulus of left Wolffian body
17, lowest glomerulus of right side

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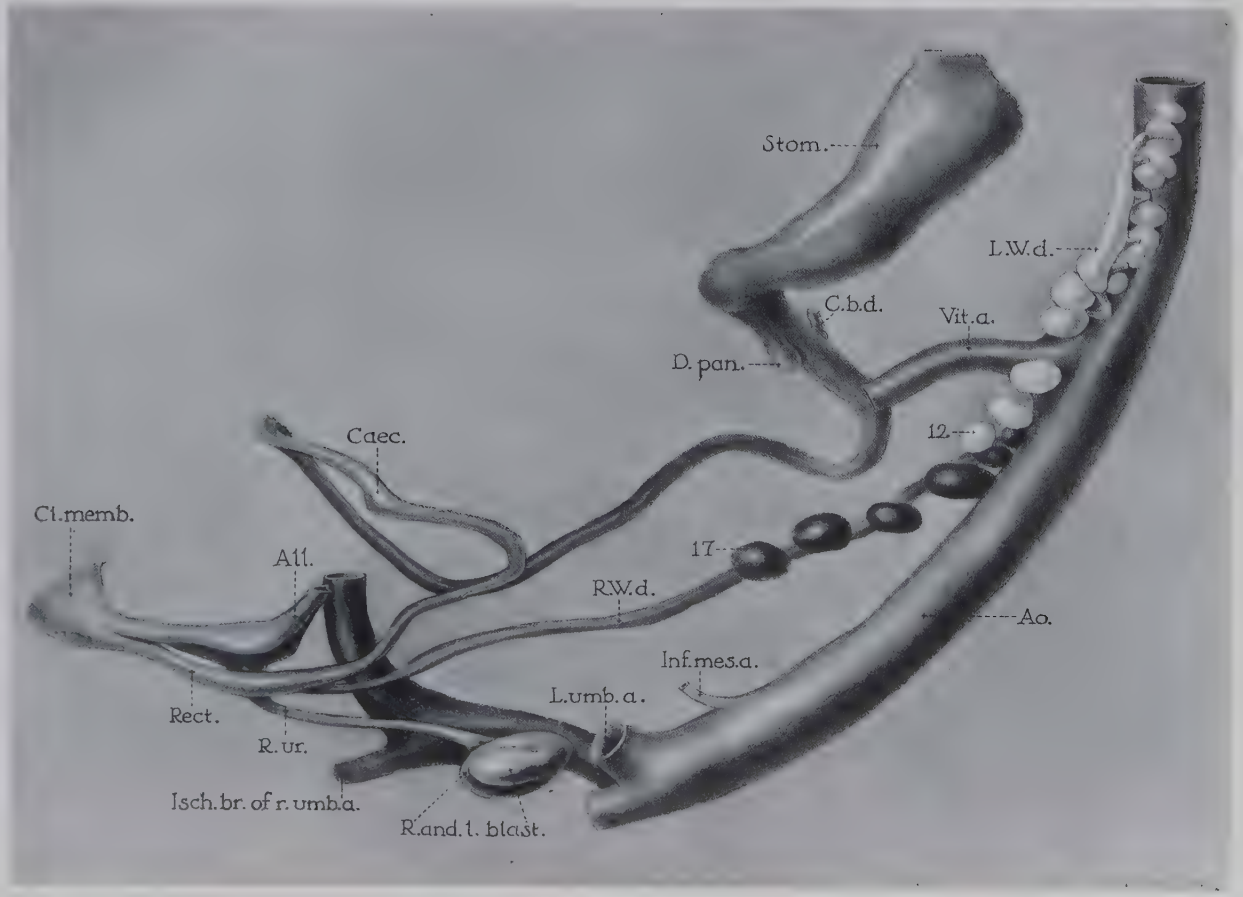


PLATE 2

EXPLANATION OF FIGURE

7 Wax reconstruction of blood vessels and kidneys of human embryo shown in plate 1, seen in ventral view. × 55. Note absence of left ureter, fusion of left and right blastemas, the more acute angle at which the left umbilical artery leaves the aorta, the greater size of the left posterior cardinal vein, and the actual contact of blastemas with the second portion of the posterior cardinal veins (compare *Pc.2*, fig. 1; *V.*, fig. 12).

ABBREVIATIONS

Caud.a., caudal artery
Il.anast., anastomosis between posterior cardinal veins in iliac region
L.med.caud.v., left median caudal vein
L.v.com., left vena comitans (compare fig. 1)
R.isch.v., right ischiatic vein
R.post.card.v., right posterior cardinal vein

(Other legends as in plate 1)

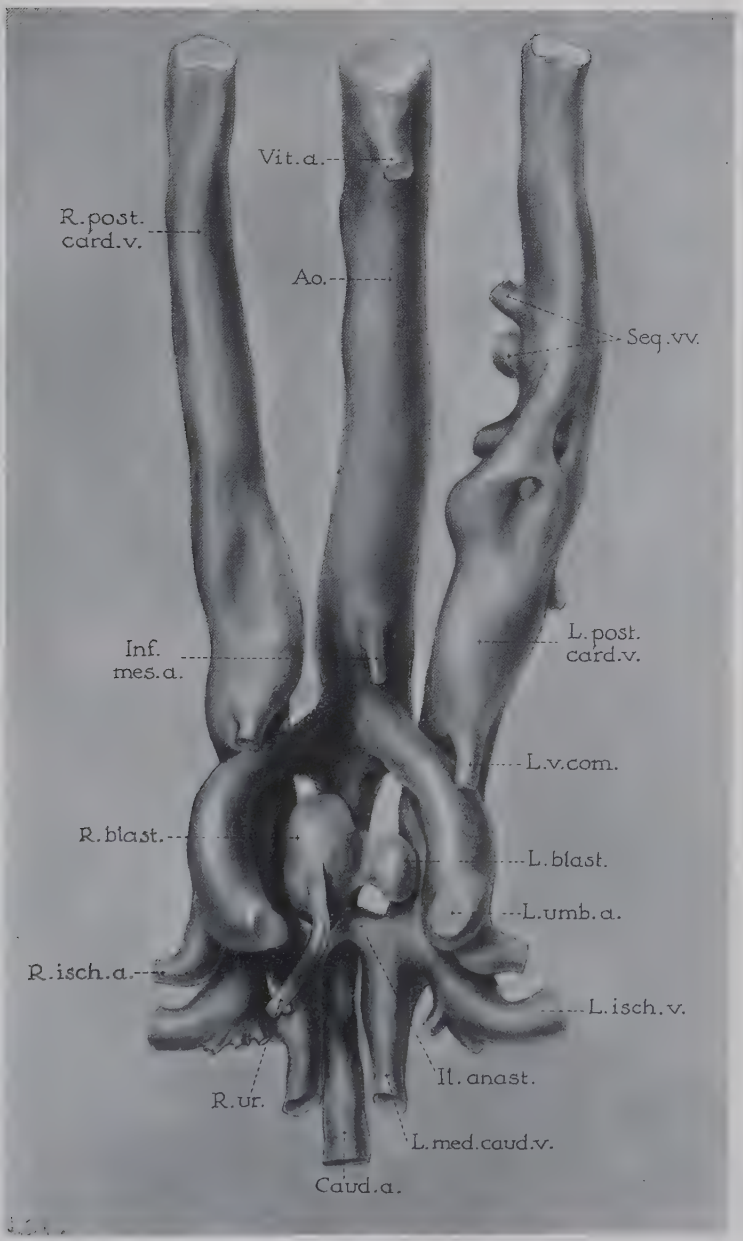

Vit.a.
R.post.
card.v.
Ao.
Seq.vv.
L.post.
card.v.
Inf.
mes.a.
L.v.com.
R.blast.
L.blast.
L.umb.a.
R.isch.a.
L.isch.v.
It.anast.
R.ur.
L.med.caud.v.
Caud.a.

PLATE 3

EXPLANATION OF FIGURES

Figures 8 and 9. Bladder of man seventy-two years of age with absence of the left kidney. × ¾.

8 Posterior view, illustrating absence of left ureter, ductus deferens, and seminal vesicle. Note dilatation of right ureter (*R.ur.*); and median spur at prostate gland.

9 Interior of same bladder, showing single ureteral orifice (arrow indicates opening of right ureter), the trabeculated inner surface of bladder, and the embryonic character of the urachus.

Figures 10 to 12. Cross-sections (20 μ) of 10-mm. human embryo shown in plates 1 and 2. × 39.

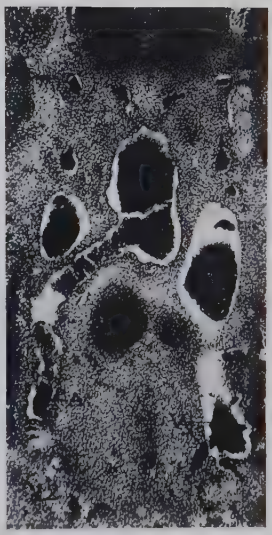
10 Section 339, at level of sixth mesonephric glomerulus of left side (compare pl. 1); illustrating dilatation of left Wolffian duct (*L.*).

11 Section 378, showing left Wolffian body (*W.d.*) devoid of tubules, but containing enlarged posterior cardinal vein. *14*, fourteenth mesonephric glomerulus of right side (compare pl. 1).

12 Section 425, at level of umbilical crotch. *A.*, right umbilical artery; *V.*, left posterior cardinal vein. The section passes through the ventral surfaces of the blastemas just before they fuse with each other (compare fig. 1 and pls. 1 and 2).

R.
ur.
8

9



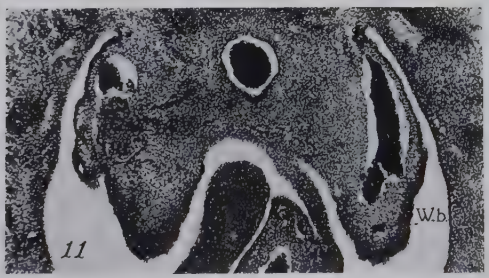

W.b
11

PLATE 4

EXPLANATION OF FIGURES

Transverse sections through chick embryos in which interruption of the Wolffian ducts has resulted in agenesis of the ureter, but persistence of the blastema.

Figures 13 to 15. Three sections through embryo 21 (compare fig. 2). × 30.

14 Section at level of umbilical crotch. Arrow at left indicates right ureter; arrow at right, narrow portion of left blastema.

13 Section above level of umbilical arteries, showing large blastema on either side of aorta (*Bl.*) and lower end of hydronephrotic left Wolffian duct (*W.*).

15 Section below level of umbilical arteries, showing difference in density of left blastema (*Bl*) containing no ureter, and of right blastema, which is being organized by the ureter.

16 Section through embryo no. 215 (compare figs. 3 and 21). × 30. Shows expansion of renal blastemas (*Bl.*) above level of umbilical crotch, in absence of both ureters. Note empty Wolffian bodies.

17 Section through embryo no. 231 (compare figs. 4 and 22). × 36. Arrows indicate right and left blastema being compressed by umbilical arteries, but not as much as in older stages.

18 Section through embryo no. 174 (compare fig. 23). × 18. Right Wolffian body is normal. Left Wolffian duct interrupted at level of thirtieth somite (table 1). Note enormously distended left Wolffian duct (*Hyd.W.d.*), which has prevented formation of left blastema at this level, between duct and left cardinal vein. Ureter present on right side (*R.ur.*). Arrow indicates hydronephrotic glomerulus. (See p. 335.)

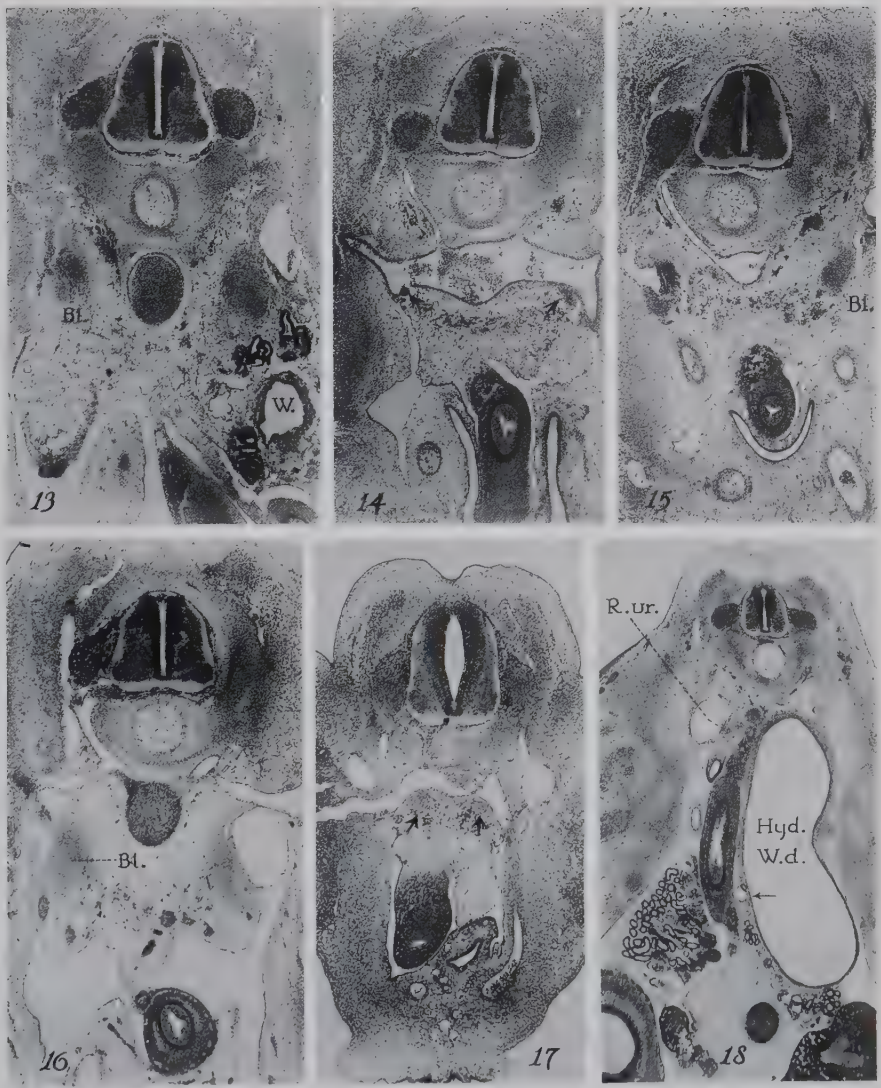

Bl.
W.
13
14
Bl.
15
Bl.
16
17
R.ur.
Hyd.
W.d.
18

PLATE 5

EXPLANATION OF FIGURES

Chick embryos incubated from two to four days after the Wolffian ducts had been interrupted experimentally. × 5.

19 and 20 Embryo no. 190 (six days). Both Wolffian ducts stopped terminally at level of twenty-ninth somite. Note abortive allantois and leg bud cleft by operation (compare fig. 5).

21 Embryo no. 215 (five days thirteen hours). Both Wolffian ducts stopped terminally, at level of nineteenth somite. Note wing buds cleft by operation (compare figs. 3 and 16).

22 Embryo no. 231 (four days fourteen hours). Both Wolffian ducts stopped terminally at level of twenty-fifth somite. Lesion severed spinal cord. See notch in back at level of leg bud. Note rudimentary allantois (compare figs. 4 and 17).

23 Embryo no. 174 (six days one hour). Right Wolffian duct stopped terminally at level of thirtieth somite. Note leg bud cleft by operation (compare fig. 18).

19
20
21
22
23

A PRELIMINARY STUDY OF THE DISTRIBUTION AND CHANGES IN THE INORGANIC SALTS DURING EMBRYONIC DEVELOPMENT OF THE CHICK[1]

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THREE PLATES (NINE FIGURES)

A great deal of literature has been compiled dealing with the morphological aspects of the developing organism, and yet up to the present time very little is known concerning the fundamental mechanism of differentiation finally resulting in the formation of a community of cells possessing varying functional activities. Therefore, the necessity of tackling this problem from an entirely new aspect was realized, and in order to determine the relation of various factors with differentiation and growth, it was deemed advisable to observe the changes, orientation, and distribution of the mineral salts during the embryonic development of the chick. For this investigation the micro-incineration method of Policard ('29, '30, '31), recently improved and applied to cytological problems by Scott ('30 a and '30 b), was employed. These preliminary histochemical studies yielded promising results, as it was clearly demonstrated that a definite migration of the inorganic radicals occurs during embryonic development, while, on the other hand, the deposition of the mineral salts in normal adult tissue remains relatively fixed. Whether such a phenomenon furnishes a reliable key to the explanation remains to be proved, but, nevertheless, the results obtained

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in this preliminary study are suggestive and appear to be a move in the right direction.

MATERIAL AND TECHNIQUE

Chick embryos of fifty-five hours, four and one-half days, and seven days incubation were fixed whole in a mixture of nine parts of absolute alcohol to one part of neutral formalin, as it was found that the above fixing fluid neither added to nor detracted from the mineral elements of the cells, and at the same time gave excellent fixation and yet caused no detectable difference in the ash deposits. After fixation for twenty-four hours, the embryos were passed through many changes of absolute alcohol in order to insure complete dehydration and to remove the formalin. They were then cleared in xylol, embedded in paraffin, and sectioned at 5 μ. In order to avoid any contact with water, the sectioned material was smoothed out upon the glass slide with the aid of a drop of absolute alcohol. The preparations were then incinerated in a special electric quartz oven at temperatures varying from 625° to 650°C. for periods ranging from twenty-five to forty-five minutes. The incinerated sections were mounted in the dry manner and studied under the microscope with dark-field illumination obtained by employing a Zeiss cardioid condenser. Every other section was used as a control, after treatment with the above fixative and staining in Harris's hematoxylin and eosin. A comparative examination of the inorganic structure of the incinerated with the control sections shows that embryonic material lends itself beautifully to this technique, especially as little or no shrinkage is detectable (photomicrographs 1 and 2). All the inorganic salts common to the organism and which are not volatilized by the heat of incineration remain in their original position. Iron, when present, assumes the form of iron oxide and is easily recognized by its color. Calcium is, in all probability, present as an oxide. Silicates are recognizable because of their birefringence when viewed with polarized light.

Policard has suggested that the light bluish ash indicates the presence of an increased amount of sodium. In the description which follows we have made use of this observation as a possible indication, in organs and in gross parts of organs, of the presence of this element in relatively large amounts. He also contends that tissues containing large amounts of phosphorus are incinerated only with difficulty. It is quite possible to differentiate clearly between thin incinerated films of calcium carbonate and of calcium phosphate by the color and refringence of the ash. The calcium carbonate is bluish white and very finely divided; the calcium phosphate is quite white and in coarser aggregations which reflect large amounts of light from their surfaces. Whether this finding can be applied to the study of the tissue ash in sections is of course highly problematical.

OBSERVATIONS AND DISCUSSION

The inorganic structure of the embryo is beautifully depicted by dark-field illumination, and an examination of the micro-incinerated sections reveals the fact that the tissues of the organism retain their individual, morphological, and histological characters as the mineral ash deposit bears the same topographical relationship when compared with the control preparations.

One great advantage of this technique, and also a very striking and distinctive feature, is that the concentration and orientation of the inorganic salts varies considerably within the different cell layers of the developing embryos, as is demonstrated by the accompanying series of photomicrographs. A comparison of the inorganic remains of the organism during growth and development will now be described within the fifty-five hours' and the four and one-half days' embryonic chicks.

The amnion, ectoderm, and mesoderm of the fifty-five hours' chicken can be distinguished from each other by the enormous variation in the arrangement and deposition of the mineral salts. The amnion, which consists of a distinctive concentra-

tion of calcium ash, contrasts greatly with the mesoderm. Within this layer the inorganic salts are solely confined to the cells themselves, the tissue spaces being entirely devoid of ash. The nuclei are well delineated by a mixture of calcium and iron oxide in the chromatin material, while the concentration of mineral deposit in the general protoplasm is very diffuse, and unlike that in the nuclei contains no detectable trace of iron oxide; the cytosome appears to consist mainly of calcium. Occasionally cell processes can readily be distinguished in which the inorganic salts assume a distinctive orientation.

The ectoderm is also characterized by a distinct concentration of ash; most of it is calcium, with probably some sodium present which gives a whitish blue color (Policard). These salts are well distributed in the cytoplasm and also in the nuclei, which contain less iron than the nuclei of the adjacent mesodermal cells. No visible increase or change in orientation in any one of the mineral salts between the fifty-five hours' and the four and one-half days' embryo could be detected within these layers.

The developing brain. The changes in distribution of the mineral salts in the developing brain of the embryo are very significant. The most noticeable and marked feature during growth of this organ is the gradual increase in the calcium content which is accomplished by a less conspicuous decrease in the amounts of iron oxide. A study of the fifty-five hours' embryonic brain clearly indicates that both these substances are present in a minor degree and appear to be more or less evenly distributed throughout the walls, forming the diencephalon, mesencephalon, and metencephalon.

Comparative observations of the inorganic structure of the brain at the several phases of development show that the younger embryonic material incinerated less readily than the older. This phenomenon is probably due to the smaller amounts of calcium present in the younger brain, together with the greater quantities of phosphorus. An examination of the accompanying photomicrographs of the fifty-five hours'

and four and one-half days' material demonstrated the visible outstanding increase in the calcium content of the latter (figs. 5 and 6). Observations of a large series of micro-incinerated brains indicate that growth in this organ is inorganically expressed by a gradual increase in calcium and a decrease in iron salts. The fluid which fills the brain cavity leaves a considerable unorganized calcium ash (fig. 6).

In the *neural tube* of the four and one-half days' chick there is a definite bluish white ash concentration along its external border. Immediately adjacent to it is the dead white of calcium crystals for an area perhaps double that of the peripheral layer. The central canal is lined by typically elongated cells which show a marked difference in their mineral deposit. Those in the dorsal portion of the tube (especially next to the lumen) contain iron and phosphorus in greater quantities than do those in the ventral part. The cells of the ventral portion of the tube give the dead white ash indicating the presence of calcium. The lumen of the central canal is conspicuous by the presence of small amounts of mineral salts. The upper half of this ash shows more iron oxide than is in the lower part of the lumen.

The inorganic remains of the anterior-horn cells are clearly detected and consist of relatively large amounts of iron and calcium, with a greater concentration of iron oxide within their nuclei. The periphery of the cord shows no cellular structures and is characterized by an extremely delicate bluish ash (which Policard contends represents sodium) following the general course of the nerve fibers, and at the same time assuming a distinct orientation, as is shown in figure 8.

The calcium content in the neural tube, as in the brain, increases gradually with the development of the organism, as a comparison with the later stages of growth demonstrated.

The notochord of the four and one-half days' chick shows a reticulated ash in the center, while the interspaces of the cells are entirely devoid of the mineral salts. The elongated precartilage cells, which surround the notochord, stand out in sharp contrast (fig. 8). The precartilage tissue leaves an

enormous calcium deposit which is so concentrated that it obliterates the individuality of the cells. It is of interest to note that these structures appear to contain no trace of iron salts. Comparative examinations of the mineral content of the precartilage cells of the fifty-five hours' and seven days' embryo clearly indicate that the four and one-half days' chick contains less calcium than the seven days' one and considerably more than the fifty-five hours' (figs. 2, 3, 4, and 8).

Rathke's pouch. Figure 5 shows a section through the forebrain and Rathke's pouch which includes the roof of the pharynx. The forebrain is in no way different from others described, it being distinctly cellular with a large amount of iron oxide present, and is limited in this case very clearly by the delicate band of condensed tissue which will later form the meninges.

The upward extension of the roof of the pharynx is characterized by a definite localization of mineral deposit. At this stage the cells have differentiated somewhat from the pharyngeal lining and contain a visibly greater quantity of inorganic salts. This deposit is clear white as compared with the yellowish color of the forebrain, which is due apparently to the presence of iron oxide. It is therefore again apparent that an early chemical differentiation of tissue is brought to light by this method of micro-incineration. The inorganic remains are visibly separated from the surrounding mesenchyme, not only by the differences in the amount of ash present, but also by the orientation of the salts, as an examination of the photomicrograph will disclose (fig. 5).

Developing heart. The muscle wall of the developing heart of the four and one-half days' embryo presents an inorganic structure similar to that of the mesoderm, except that there are indications that these cells forming the inner lining of the organ leave behind a bluish ash which may suggest either the presence of calcium carbonate or of sodium.

The mineral content of the endothelial cells forming the walls of the blood vessels is quite different from that of the surrounding mesenchyme. These elements are rendered con-

spicuous by the large amounts of calcium which they possess. Comparative observations indicate that their nuclei contain visibly smaller quantities of iron oxide, and in this way differ from the nuclei of the mesenchymal structures. On the whole the endothelial cells present a less organized appearance than the latter elements, this being mainly due to a difference in the aggregation and orientation of the mineral salts. The blood elements, which lend themselves beautifully to micro-incineration, exhibit less iron oxide at this stage than in older embryos.

The developing eye. In the fifty-five-hour chick the superficial ectoderm is represented by a concentration of a pure calcium ash and stands out in contrast with the lens and other developing tissues forming the eye. The incinerated lens yields a yellowish mineral deposit, suggesting the presence of a considerable amount of iron oxide together with calcium. The inorganic structure of the retinal layer of the optic cup is made up of more calcium than the adjacent pigmented layer, both of which contain quantities of iron oxide. In the former layer there is a slight tendency for the calcium to aggregate along the inner wall forming the posterior chamber, and an examination of the more advanced stages of development shows that a definite migration of the mineral salts, especially calcium, has occurred. In the seventh-day embryo there is a marked orientation of mineral deposit. A similar migration of inorganic salts is observed to occur during growth of the lens. In the fifty-five-hour chick the mineral constituents appear to be evenly distributed, while in the fourth-day one an increase in the calcium content is clearly visible, and by the end of the seventh day there is an appreciable difference in the ash deposit. This interesting phenomenon suggests that a definite migration of the mineral salts occurs during embryological development. The white calcium ash has accumulated along the inner cavity of the lens, while the iron salts appear to have emigrated toward the periphery. The inorganic structure of the iris is also clearly shown by micro-incineration. The free end adjacent to the

lens is conspicuous by a dense aggregation of calcium deposit, and contrasts with the surface facing the cornea, which consists of a diffuse bluish ash, suggestive of sodium. The leucocytes of the intervascular space of the iris reveal a well-differentiated mineral ash. The fluids of the anterior and posterior chambers leave a curious inorganic deposit.

Distribution and changes in inorganic salts of seven-day-old embryo

Brain. The brain at this period incinerates better than in the younger stages, owing to the appreciable increase in its calcium content and apparent decrease in phosphorus. The inorganic salts as a whole have assumed a more definite organization, which becomes most conspicuous when compared with the developing brain of the younger embryos. There is also a noticeable decrease in the iron salts. The inorganic structure of the well-formed nerve fibers is revealed in the form of a definite orientation of mineral deposit. The optic radiation shows up with extraordinary clearness, exhibiting a distinctive calcium deposit—rather more than in the adjacent tissue.

Spinal cord, posterior. The lining of the central canal shows less calcium and more iron than did the ventricular lining of the brain (fig. 2). The ciliated cells lining the central canal show extraordinarily well. There is a fine beaded ash deposit representing each cilium. The peripheral portion of the cord gives a non-cellular evenly distributed white deposit of ash. The ventral portion of the cord shows an ash which is orientated vertically. The laminae of the vertebrae are especially well marked, for the cells lining their under surfaces (i.e., in the perichondrium and adjacent matrix) show an entirely different ash deposit than do the adjoining mesenchymal cells. Here there is a marked concentration of calcium and iron salts (fig. 2).

Spinal ganglia. The spinal ganglia first show as whorls of cells, the cytoplasm of which contains a rather considerable amount of calcium ash. The dorsal root is probably greater in inorganic salt content than the ventral (fig. 2).

Notochord. The central portion of the notochord contains cells which show every sign of degeneration (figs. 2 and 3). The nuclei are quite prominent. Indeed, the ash almost resembles that of degenerating lymphocytes (probably pycnotic nuclei). The cytoplasm is very stringy and vacuolated, there is no ash deposit in the interstitial spaces. There is a remarkable concentration of calcium ash about the sharply delineated older portions of the notochord. These cells are precartilage and possess more individuality than in the four and one-half days' embryo, owing to a more definite orientation of the mineral salts (figs. 3 and 8). There is so much clear white ash distributed evenly throughout the cells that it is impossible to see the nucleus which ordinarily shows up very clearly. This area of precartilage cells is about ten to fifteen cells thick. The concentration of ash in the precartilage cells is greatest on the dorsal half of the notochord. The amount of iron in some of the cells immediately adjacent to the old portion of the notochord is rather less than in those of the periphery.

Mesoderm and blood vessels. The mesoderm cells are more evenly distributed; there is more definite organization and concentration of calcium in the nuclei. No visible difference in mineral content and distribution can be detected between the mesodermal structures of the four and one-half and seven days' embryos. There is considerably more mineral ash deposit in the cytoplasm of the endothelial cells than in the adjacent mesenchyme (fig. 4). Blood vessels contain immature cells which show considerably more iron oxide than is in the wall of the blood vessels themselves.

Mesenchyme. Two types of cells are clearly depicted: one, in which the nucleus is highly refractile and contains large quantities of calcium and iron oxide; another, in which the nucleus is lightly marked out and the ash is non-refractile. The latter cells are bluish white, which may indicate the presence of an increased amount of sodium.

Somites. There is a most striking difference between the somites and the surrounding area. The nuclei of the cells

constituting the somites are beautifully defined. The cytoplasm contains a large quantity of calcium ash. There is also a considerable amount of iron oxide distributed in the nuclei and cytoplasm, but more in the nucleus. The cells which are about to take part in the formation of somites contain a larger quantity of ash than those in the surrounding areas and are clearly defined (fig. 7).

Mesonephros. The mesonephros at this stage shows well-differentiated convoluted tubules and collecting ducts. Examination of the inorganic residue shows a visible difference in the two types of tubules, which awaits detailed study. It is significant that there is no ash whatever in Bowman's capsule. In general, the mesonephric ash is mostly calcium (fig. 7).

Lung. The tissues forming this organ lend themselves very well to micro-incineration. No apparent shrinkage can be detected when compared with the control preparations and the whole inorganic skeleton is clearly revealed. The bronchi are rendered very conspicuous on account of the appreciable amounts of iron oxide present, and owing to this fact they are inorganically differentiated from the remaining structures which consist chiefly of calcium (fig. 9).

Hind limb. There is visible a group of precartilage cells which resemble in general those around the notochord. In the center of this cell group there are cells which may be cartilage. They contain rather less iron oxide and more calcium than do their neighboring elements. The mesenchyme is somewhat condensed around the developing limb. The mineral salts are so concentrated in the ectoderm that the individuality of the cells composing the layer is entirely lost. The remaining mineral deposit appears to contain calcium and is devoid of any trace of iron oxide.

SUMMARY

A consideration of the changes, distribution, and orientation of the inorganic salts during growth, although in the nature of a preliminary study, has brought to light certain

interesting and fundamental facts. Apart from demonstrating the inorganic structure of the growing organism, these histochemical observations have revealed that embryonic development is inorganically expressed by an increase in certain mineral salts and a decrease in others. This phenomenon has been admirably shown, for example, during growth of the brain, where a gradual increase in the calcium content is accompanied by a decrease in the iron salts. And in the case of other organs, during certain phases of growth, a definite migration of the inorganic constituents has been established. When this behavior is correlated with the fact that morphogenesis of the embryonic chick is accompanied by apparent chemical differentiation of the inorganic constituents, and especially as the proportions of the mineral salts in the fully differentiated adult tissue remain fixed, it is suggestive that the inorganic radicals play a fundamental rôle in cell differentiation.

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EXPLANATION OF PLATES

All photomicrographs, except figure 1, represent micro-incinerated sections of chick embryos of varying stages of development.

PLATE 1

EXPLANATION OF FIGURES

1 Control preparation of longitudinal oblique section cut through the posterior region of a seven days' embryonic chick. Note the appearances of neural tube, the laminae and basal portion of the spinous process of vertebra, notochord, and ganglia, and compare with the micro-incinerated section depicted in figure 2.

2 Showing the inorganic structure of the neural tube, ganglia, and notochord. Note the concentration of calcium oxide in the laminae and basal portion of the spinous process of vertebra as well as in the precartilage cells surrounding the notochord and observe the internal remains of the latter structure.

3 The same, at a higher magnification. Note the peripheral ventral portion of the cord which shows an orientated calcium-oxide ash.

4 Transverse section through notochord of a seven days' embryo, depicting the inorganic remains of precartilage structures, which are composed of enormous concentrations of pure calcium ash.

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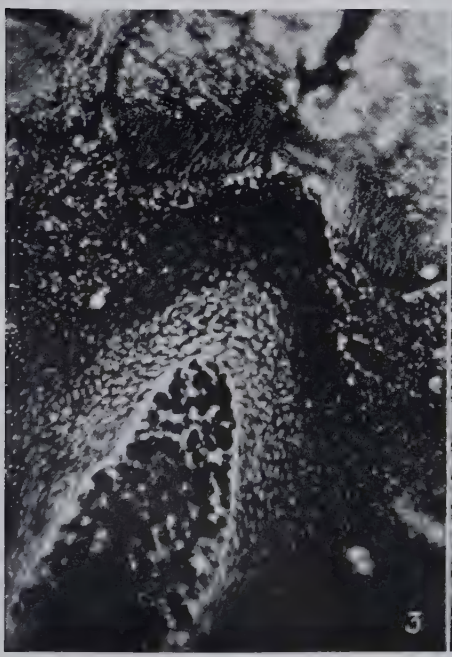
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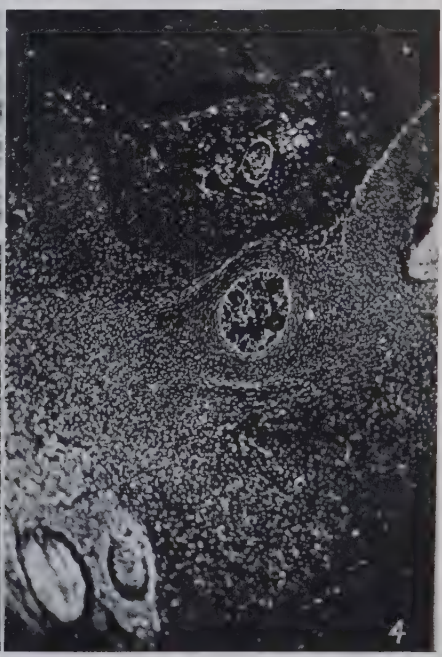
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PLATE 2

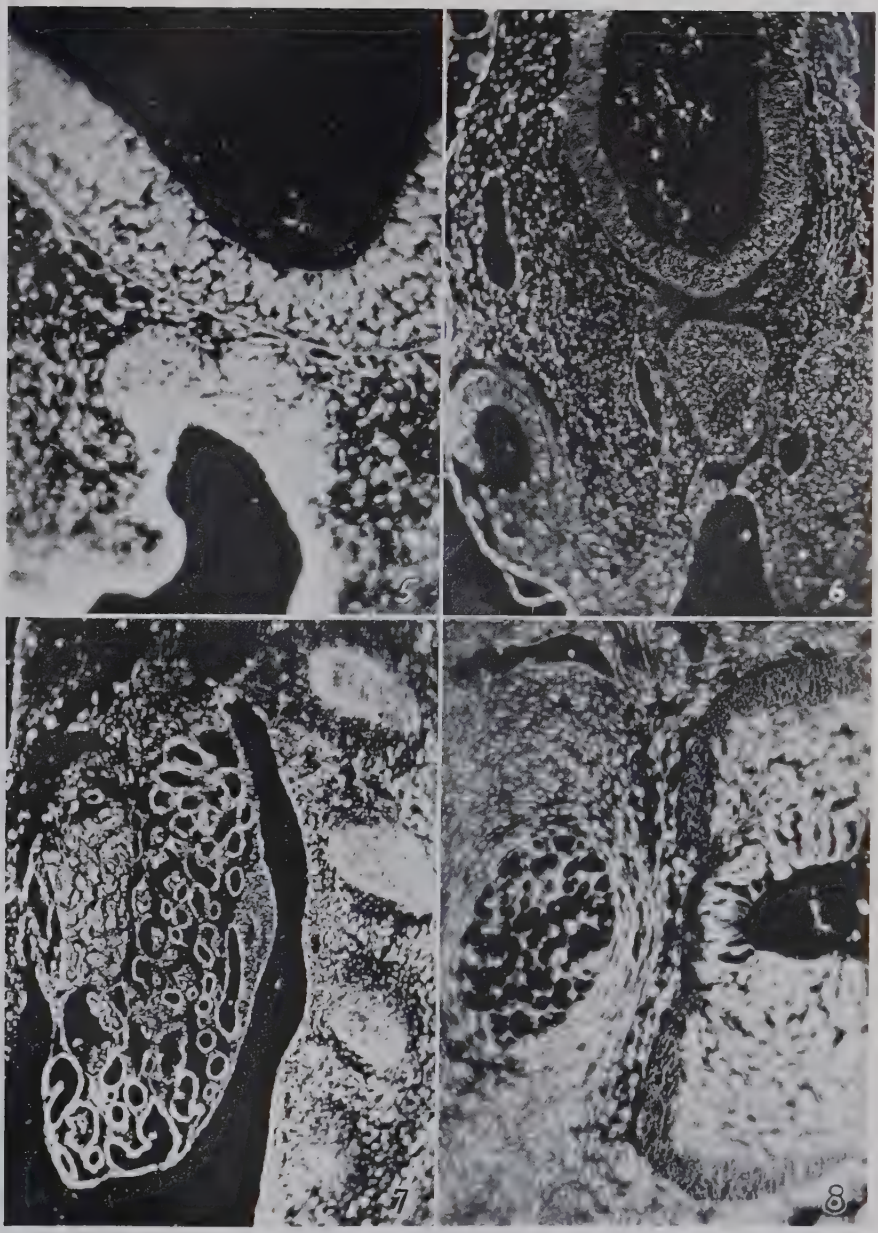
EXPLANATION OF FIGURES

5 Oblique section through the forebrain and Rathke's pouch in a four and one-half days' embryo, showing the increase in calcium content and decrease in iron oxide as compared with a fifty-five hours' embryo depicted in figure 6.

6 Showing forebrain of fifty-five hours' embryo; compare inorganic remains with that illustrated in figure 5.

7 Micro-incinerated mesonephros of a seven days' embryo. Also note difference in mineral deposit between the tubules and surrounding tissue.

8 Transverse section through posterior region of four and one-half days' embryo, showing definite orientation of inorganic salts in peripheral region on neural tube. The developing notochord and surrounding precartilage cells are well defined.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9 Incinerated section through lung of seven days' embryo. The concentrated orientation of mineral deposit around the bronchi represents iron oxide; the remainder consists of calcium salts.

HEMOPOIETIC LOCI IN NECTURUS MACULOSUS

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ONE PLATE (SIX FIGURES)

Centers of hemopoietic activity in vertebrates are found associated with many different organs, and in both ontogeny and phylogeny there have occurred extensive shifts in the location of such centers as well as changes in the types of blood cell produced. The evolutionary history of the hemocytopoietic tissues reveals rather definite trends of differentiation which are to a certain extent also recapitulated in the ontogeny of the vertebrates.

As pointed out by Jordan and Speidel ('30, '31) the myeloid tissue (spleen) of Myxine is aggregated in the spiral valve, but in the lungfish it is sharply segregated in the stomach wall, while in the elasmobranchs it appears as a separate organ outside the gastro-intestinal tract. In the elasmobranchs the ovaries and testes may also function as a granulocytopoietic organs, and in many fishes the intertubular tissue of the mesonephros in addition is hemocytopoietically active. Besides these more common loci of hemopoiesis in fishes there are also other aggregates of hemopoietic tissue: in the wall of the esophagus of elasmobranchs, in the epicardium and cranial capsules of the Chondrostei, and in the intrahepatic pancreatic tissue of certain teleosts.

In the higher urodeles (salamanders) granulocytopoiesis is restricted to the periphery of the liver, and erythrocytopoiesis and thrombocytopoiesis take place in the spleen (Jordan and Speidel, '24). In Proteus, however (Drzewina, '05), renal hemopoiesis is present and the interior as well as

the periphery of the liver is involved in granulopoiesis.[1] In the Anura there is at metamorphosis a shift of the hemopoietic locus from the mesonephros, the larval hemopoietic organ, to the spleen, the adult hemopoietic organ (Jordan and Speidel, '23 a). Again in the mammals the yolk sac, liver, and spleen are usually active hemopoietically in the embryo, while in the adult the bone-marrow and lymph nodes become the important sites of blood formation.

Since important changes in hemopoiesis do occur both in phylogeny and ontogeny, it seemed of interest to make a survey of hemopoiesis in Necturus, which is permanently larval or neotenous and is also generally regarded as a relatively primitive urodele. The determination of the relative level of development attained by certain families of urodeles, i.e., whether they are juvenile rather than primitive, is sometimes difficult, due largely to the fact that some fail to metamorphose while others metamorphose incompletely. In addition, in incomplete metamorphosis certain tissues and organs show varying degrees of transformation. Consequently, no one criterion can be safely used as a measure of metamorphosis, but all the changes must be viewed as a whole if a proper estimate is to be made of the degree of ontogenetic differentiation attained by a given species. It is hoped that the present study, which forms a part of a more extensive survey of hemopoietic loci in non-metamorphosing and metamorphosing urodeles, may furnish an additional criterion for a such a determination.

The major hemopoietic loci, spleen, liver, mesonephros, epicardium, thymus, and fat body of the caudal skeletogenous septum, will be described in the order given. A detailed discussion of the nature of the ancestral blood cell, the hemocytoblast, is reserved for a later paper dealing with blood formation, following lead poisoning, in normal and splenectomized animals.

[1] According to Jordan ('32), Proteus anguineus lacks a hepatic, subcapsular, lymphogranulocytopoietic layer and the mesonephros constitutes the sole organ of eosinophile and neutrophile production. (Jordan, H. E., 1932. The lymphocytes of the urodele, Proteus anguineus. Anat. Rec., vol. 52, p. 21, abstract.)

DESCRIPTION

Spleen

Necturus possesses a typical elongated urodele spleen with a rather delicate capsule in which smooth muscle fibers are lacking and no heavy trabeculae extend from the capsule into the interior. Definitive arteries are few and exhibit no characteristic pattern of distribution. Veins appear to be more numerous. Both vessels have inconspicuous walls and possess a minimum of smooth muscle. The smaller arterial terminations may be recognized by the more or less concentric arrangement of fibroblasts in their immediate vicinity. The veins, however, end with simple endothelial walls, about which little differentiation of connective tissue may be distinguished. The lumina of the venous sinusoids are continuous with the intercellular spaces of the reticular stroma.

The meshes of the reticulum are usually closely packed with free cells, lymphoid cells, erythroblasts, erythrocytes, thromboblasts, and thrombocytes. There is no sharp segregation of red and white pulp. The amount of lymphoid tissue is extremely variable. In certain spleens the lymphoid tissue predominates locally (fig. 5), forming irregular masses and cords, but no definite splenic nodules are present. Occasionally neutrophiles and eosinophiles are seen, but these are probably migratory cells and have not differentiated in situ, although Hartmann ('26) finds that granulocytes are differentiated in the spleen of axolotl.

Erythrocytophages are always present and usually numerous, but the numbers found vary considerably, depending upon the season and the previous experiences of the animal. These phagocytes arise chiefly within the spleen from reticular and lymphoid cells. Some, however, may accumulate in the spleen from the circulating blood, since intravascular phagocytosis of erythrocytes is common under such experimental conditions as lead poisoning (Dawson, '30 a). Pigmented cells, representing terminal stages of erythrophagocytosis, are also present.

The predominant function of the spleen is the production of erythrocytes and thrombocytes. The lymphoid hemoblasts of the spleen apparently differentiate for the most part in situ, but many immature erythrocytes are normally present in the circulating blood and may frequently multiply there by mitosis (Dawson, '30 b).

Liver

Although the amount of hemopoietic tissue in the liver is relatively small compared with the amount of hepatic parenchyma, still the very large size of this organ in Necturus permits of the accumulation of considerable blood-forming tissue. The total mass of hemopoietic tissue is probably much greater than the volume of the spleen. The distribution of hemopoietic tissue in the liver of Necturus is very similar to that of Proteus (Drzewina, '05). The perihepatic capsular layer is present, but not highly developed (figs. 1, 2). At the hilus there are frequently small masses of intrahepatic pancreatic tissue, and in this location there is a considerable massing of hemopoietic tissue. From the hilus this tissue spreads throughout the entire liver, following the course of the portal canals (portal veins and bile ducts) which it ensheaths more or less completely (fig. 1). In addition, in many animals there occur also numerous isolated lymphoid masses interposed in the hepatic parenchyma (fig. 3). They vary considerably in size and number and may be or may not be associated with pigmented phagocytes.

All the blood-forming tissue of the liver is essentially granulocytopoietic. Neutrophiles, eosinophiles, and large and small lymphoid cells are present. There is a preponderance of neutrophiles, while eosinophiles are relatively sparse. There is no evidence of erythrocytopoiesis or thrombocytopoiesis in the lymphoid tissue, but in the liver sinusoids erythroblasts in various stages of differentiation are frequently observed. Such cells probably did not differentiate from hepatic lymphoid tissue, but were carried to the liver by way of the circulation.

Mesonephros

The distribution of intertubular lymphoid tissue in the mesonephros of Necturus has been described by Chase ('23), who pointed out that it is very similar to that of Proteus (Drzewina, '03, '05). The lymphoid tissue is rather uniformly distributed throughout the entire length of the organ except that in males it is greatly reduced in the anterior or genital portion. In cross-sections of the mesonephros the intertubular tissue is seen to invest all portions of the uriniferous tubules (fig. 6) except the distal convoluted (striated) portions which occupy the so-called central zone of the gland, adjacent to the postcava. The distal convoluted tubules are separated from the sinusoidal efferent veins by only an inconspicuous connective-tissue capsule.

As in the liver, the intertubular tissue of the mesonephros is predominantly granulocytopoietic and the proportions of lymphoid cells, neutrophiles, and eosinophiles are quite similar. Mitoses of immature cells are frequently observed. Monocytes are also conspicuous in these areas. They frequently contain ingested material and are often seen migrating through the walls of the mesonephric tubules. Some doubtlessly enter from the peritoneal cavity by way of the nephrostomes and others may come from the circulating blood.

Heart

Several investigators (Wituschinski, '28; Jordan and Speidel, '30, and Jolly and Lieure, '31) have described erythropoiesis as occurring within the heart of urodeles especially in the sponge-like structure formed by the muscular trabeculae of the ventricle. The hemoblasts, according to them, are derived in part at least from the endothelium, while others may accumulate in these locations from the general circulation. Sections of hearts of normal Necturus showed little intracardiac erythropoiesis, although immature cells were encountered in the spaces between the muscular columns. There was no evidence of an endothelial origin of hemoblasts.

On the other hand, a well-defined layer of granulopoietic tissue was found in the visceral pericardium covering the more muscular portions of the heart. The layer was not continuous over the ventricle, but was well developed over the bulbus (fig. 4) and was gradually reduced over the truncus, the distribution paralleling rather closely that described by Drzewina ('05) in the sturgeon. Lymphoid cells and granulocytes were the predominating blood elements in this pericardial layer. The blood supply was relatively abundant.

Thymus

These glands are found on the sides of the head, dorsal and medial to the mandibular articulation. Compared with the loci of hemopoiesis already described, these organs appear relatively unimportant in blood formation. The predominating element is the lymphoid cell, with a few scattered granulocytes. There is little evidence of granulocyte differentiation in this location and most of the cells found here probably represent migratory elements.

Fat tissue

Variable amounts of lymphoid tissue, apparently at a maximum in the spring, are associated with the several fat deposits of Necturus. No special fat body is found along the genital tract and subcutaneous fat occurs in limited amounts. The most conspicuous accumulation of fat occurs in the ventral skeletogenous septum of the tail just posterior to the cloaca. Lymphoid cells are scattered throughout this tissue and in many cases extend, in association with delicate reticular tissue, along the myosepta and out between the skeletal muscle fibers. Ventrally, near the cloaca this tissue is continuous with subcutaneous connective tissue and marked lymphoid accumulations are frequently encountered in such regions. More diffuse aggregations, mingled with varying amounts of fat, are also seen in the submucosa of the roof of the mouth, around the base of the tongue, and in the region of the glottis. In all of these locations there is practically

no granulocytopoiesis. Some of the lymphoid cells appear modified, resembling erythroblasts, but they probably represent differentiations toward the plasma type of cell.

Digestive tract

Very few leucocytes of any kind are encountered in the submucosa of the digestive tract proper. The local accumulations about the mouth, pharynx, and cloaca have already been referred to.

Bone marrow

Blood formation in the bone marrow of Necturus is negligible. In the skull, the subcutaneous connective tissue of the dorsal side of the head and the submucosa of the roof of the mouth extend inward to fill the irregular spaces not occupied by the bones of the cranium and frequently contain considerable numbers of leucocytes, but hemopoietic activity appears to be at a minimum. The bones of the cranium are joined together either by cartilage plates or rather dense fibrous connective tissue. Scattered leucocytes also occur in the vertebral canal in the loose connective tissue about the spinal cord. Within the bones, however, the leucocytes exhibit no tendency to become aggregated.

DISCUSSION

Although the hemopoietic loci of Necturus are rather widespread, nevertheless only the spleen and to some extent the general circulation are involved in the production of erythrocytes and thrombocytes. The remaining loci, some with compact, others with more diffuse tissue, are primarily concerned with lymphogranulopoiesis. Jordan ('31) regards the vertebrate spleen as the fundamental blood-forming organ. In the urodeles the function of the spleen has become restricted chiefly to erythrocyte and thrombocyte formation, while lymphogranulopoiesis may still occur in the interstitial tissue of many different organs.

Bone-marrow hemocytopoiesis apparently represents a secondary adaptation of a favorably vascularized tissue, appearing first in the Anura and persisting through the higher vertebrates. With the increased importance of bone marrow there occurs a progressive restriction of the sites of granulocytopoiesis, si that renal, hepatic, and epicardial loci eventually partially or entirely disappear. However, in some adult urodeles without bone-marrow hemocytopoiesis (Triturus; Jordan and Speidel, '24) renal blood formation has disappeared, and in the liver blood cells are formed only in a restricted subcapsular zone. The conditions in the larvae of metamorphosing urodeles have not as yet been sufficiently investigated. Accordingly, even without bone marrow some restriction in the number of blood-forming centers may occur. Whether this, in such urodeles, is correlated with the attainment of a higher evolutionary level or is the result of transformations accompanying metamorphosis (more complete ontogenetic development) cannot be decided until more information is at hand regarding hemopoiesis in the larvae of metamorphosing urodeles. It is, of course, conceivable that all urodeles do not recapitulate in their ontogeny the entire racial history of hemocytopoietic tissue. Moreover, any endeavor to ascertain the relative primitiveness of the blood-forming system of any amphibian must also taken into consideration the conditions found in ancestral forms such as fishes. Here, too, caution is necessary, since in the fishes it is not always easy to determine what constitutes adaptation within the group and what is of phylogenetic significance.

In the phylogeny of the vertebrates, the history of the spleen is clear, although in the higher vertebrates, with the increasing importance of the bone marrow, its hemocytopoietic functions are greatly restricted. In the history of the accessory hemocytopoietic centers of the kidney, liver, epicardium, and gonads there is not the same continuity of functional activity throughout the entire vertebrate group.

Renal hemocytopoiesis is common to most fishes, although it varies greatly in importance in the different groups. The

kidney is also active in blood formation in Proteus anguineus (Drzewina, '03, '05) and in the larval Anura (Jordan and Speidel, '23 b). This suggests that blood formation in the intertubular connective tissue of the mesonephros is more or less characteristic of fishes and of larval and neotenous Amphibia.

The distribution of the hemocytopoietic tissue in the liver varies in the different groups of vertebrates. In fishes with an intrahepatic pancreas the granulopoietic tissue is confined to a variable layer between the pancreatic and hepatic tissues and the pancreatic tissue frequently ensheaths the portal canals, as in the catfish. In many urodeles (Triturus viridescens, Amblystoma tigrinum, and Amphiuma; Jordan and Speidel, '24) hemopoiesis is restricted to an extensive perihepatic capsular layer, but perihepatic hemopoietic tissue does not occur in such anurans as the leopard frog and bullfrog. In Proteus (Drzewina, '05) a definite subcapsular layer is present and within the liver the lymphoid tissue also attains considerable proportions, being localized more or less abundantly about the blood vessels, but occurring in addition as isolated masses in the hepatic parenchyma. A similar distribution was observed in Siredon pisciformis, but the amount of hemopoietic tissue was less than in Proteus. Salamandra maculosa possessed a very thick peripheral layer from which prolongations extended deeply into the liver, but little scattered lymphoid tissue was observed. In Triton the distribution appears comparable to that in Triturus.

In Necturus a small amount of intrahepatic pancreatic tissue is encountered at the hilus of the liver. In this region hemopoietic tissue is abundant, and radiates from this locus throughout the liver, following the connective-tissue sheaths of the portal canals. Small isolated intertubular masses are also present. The resemblance to Proteus in these respects is striking. The periportal distribution in both these forms is suggestive of the conditions in fishes except that in Necturus the pancreas is mainly extrahepatic.

The finding of an epicardial layer of granulopoietic tissue in Necturus was a distinct surprise, since so far as the author is aware this condition has not been previously reported for urodeles—in fact, for very few vertebrates. A much more conspicuous lymphoid organ is found on the surface of the heart of certain ganoids (Drzewina, '05). It is supplied with a definite lymphatic circulation and is also the site of production of lymphocytes and granulocytes. There does not seem to be any reason for not regarding the epicardial layer of Necturus as homologous with the cardiac organ of the sturgeon, although the relative amount of blood-forming tissue in Necturus is small and no complex system of lymphatics is present.

The more diffuse aggregations of lymphogranulopoietic tissue in Necturus are usually associated with regions in which fat is stored. In amphibians fat is accumulated in various regions of the body, the most characteristic position being the special fat bodies within the abdominal cavity, anterior to the gonads. In the salamanders the tail may play an important rôle in this regard (Noble, '31), and in frogs various regions of the shoulder girdle contain conspicuous fat pads. During the spring, when the fat stores are depleted, lymphocytes and granulocytes frequently become aggregated in these regions. The accumulations of lymphoid tissue about the tongue and glottis are of the same nature. Perhaps the diffuse tissue about the cranium of Necturus can also be included in this category, although it may possibly be homologous with the pericranial lymphoid tissue of the ganoids described by Drzewina ('05). In the main the distribution of fat and associated lymphoid tissue appears to be of the nature of secondary adaptations to body structure and not to have the phylogenetic significance of the renal, hepatic, and epicardial loci.

The situation regarding hemopoiesis in Necturus may be summarized briefly. The restriction of the activity of the spleen to erythrocyte and thrombocyte formation and the development of a subcapsular granulopoietic layer in the liver

appear to be typically urodelan. On the other hand, the persistence of renal hemopoiesis, the presence of an epicardial granulopoietic layer, and the periportal distribution within the liver of blood-forming elements are characteristic of the conditions in fishes. Whether the latter conditions in Necturus are primitive or merely characteristic of urodele larvae cannot be determined until more data have been obtained on hemopoiesis in neotenous, larval, incompletely metamorphosed, and adult urodeles.

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PLATE 1

EXPLANATION OF FIGURES

All figures are photomicrographs. The magnification was varied in order to include a representative portion of the different organs in a single figure. The tissues in figures 1, 3, and 6 were prepared with Helly-hematoxylin and eosin; those in figures 2, 4, and 5, with the Helly-eosin-azur technic.

1 Section through the periphery of the liver, showing the subcapsular and periportal accumulations of lymphogranulopoietic tissue.

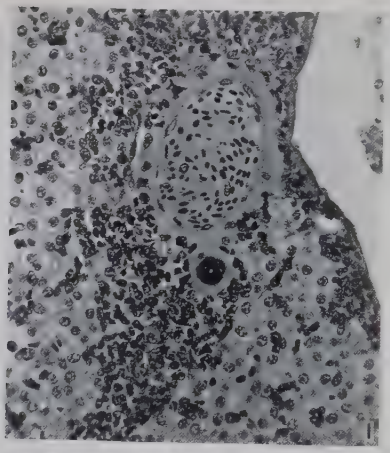
2 Subcapsular zone viewed under higher power, showing cellular elements.

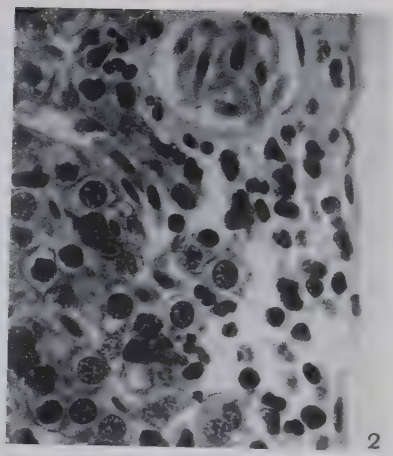
3 Section of interior of the liver, showing the isolated, intertubular groups of granulopoietic tissue.

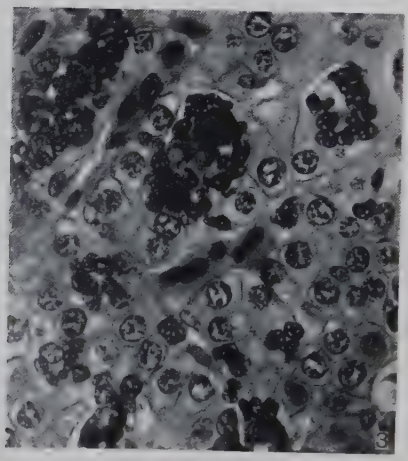
4 Portion of the wall of the conus arteriosus, showing the massing of hemopoietic tissue in connective tissue of the epicardium.

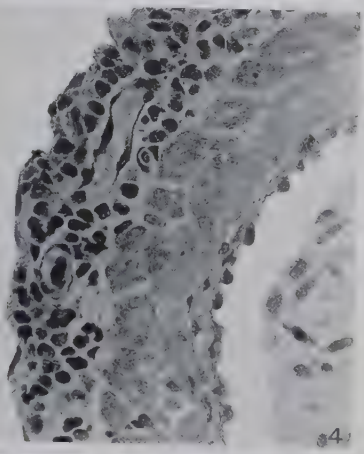
5 Small area of the spleen of an animal killed in May, showing the relative amount of red and white pulp. The lymphoid accumulations appear dark in the photograph. One artery is shown.

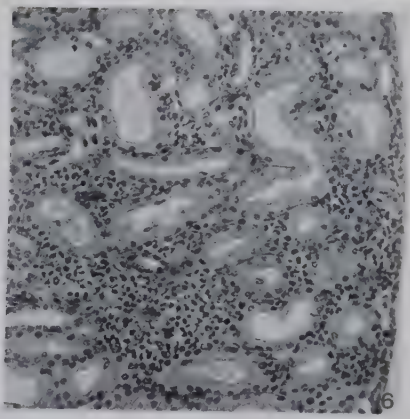
6 A section through the peripheral zone of the mesonephros, showing secretory and junctional parts of the renal tubules and the intertubular lymphogranulopoietic tissue.











PLACENTATION IN THE MARMOSET (OEDIPOMIDAS GEOFFROYI), WITH REMARKS ON TWINNING IN MONKEYS

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THREE PLATES (ELEVEN FIGURES)

MATERIAL

So little is known concerning placentation in the marmosets that a description of a well-preserved gravid uterus of one of these platyrrhine monkeys is of value. Examination of this specimen yields some interesting data concerning placentation. Reference to the scanty literature on this subject will be deferred until the description of the present specimen has been given.

The specimen was obtained through the generosity of Dr. H. C. Clark, of the Gorgas Institute, Panama. The uterus was taken from an animal captured near Panama City and killed on April 22, 1931.

GROSS OBSERVATIONS

The uterus is an oval sac approximately 5.5×3.5 cm. in greatest diameters. The tubes and ovaries are situated about equidistant from the cervix and tip of fundus. The tubes are slender and minute; the fimbriae are delicate. The ovaries are approximately equal in size and are small. They are not encapsulated. Corpora lutea are not grossly visible. The ovaries in their relative smallness differ from those of other platyrrhines in which they are conspicuously large.

The cervix and the upper part of the vagina are attached to the specimen. The interior of the vagina exhibits closely set, shaggy papillae. The external os projects, with well-

defined posterior and anterior fornices. The lips of the os are formed by delicate, rather shaggy, fimbriated projections. The cervical canal is 9 mm. long and is plugged with gelatinous mucus. The internal os is indicated by a circular muscular thickening.

On opening the uterus, twins are discovered, as is said to be the rule in marmosets (Callitrichidae). The fetuses are, respectively, approximately 47 and 44 mm. in crown-rump lengths (figs. 2 and 3). Both of the fetuses are males, with well-defined external genitalia.

The examination of the placentae and membranes has proved to be of considerable interest. The evidence from these observations leads to the conclusion that one is dealing with single-ovum twins of a hitherto unknown character, namely, monozygotic twins which have arisen within a blastocyst possessing bipolar placental attachments. Bidiscoidal placentae are normally present in many species of monkeys. Such a type of blastocyst affords opportunity for the present manner of arrangement of the fetal adnexa. In man and the great apes in which the mode of attachment of the blastocyst is quite different, or in those species of monkeys possessing a unipolar attachment, the present condition cannot occur.

In the present specimen (fig. 1) the twins lie in separate amniotic sacs, but have in common one chorion. Nevertheless there are two placental disks, one lying dorsally, the other ventrally, in the uterus. It might be supposed that a fetus belonged separately to each of the placental masses, the condition being one of double-ovum twinning. This is not the case, however. Instead, both fetuses attach by their respective umbilical cords to the same placental disk, which should be designated the primary placenta. The primary placenta is shared about equally by the circulation of the two fetuses. And, moreover, the vessels pertaining to one fetus anastomose to a considerable extent with vessels belonging to the other, so that the blood is shared by the two organisms. The opposite or secondary placental disk receives its blood supply secondarily from vessels crossing over in the membranous

chorion from the margin of the primary disk. Thus the secondary disk is shared equally by vessels from both fetuses, which anastomose likewise to some extent with one another in the secondary disk.

From the evidence that there are two amniotic sacs, but only one chorion, that the blood supplies anastomose, that the fetuses both attach to the primary placental disk, and that the fetuses are both of the same sex, we are reasonably sure that we are dealing with single-ovum twins instead of double-ovum twins.

From a knowledge of twinning in the human, the only stumbling block to such a conclusion lies in the fact that there are in the present instance two separate and distinct placental disks. This objection is readily met, however, by a consideration of placentation in monkeys in general. In the majority of platyrrhines, as well as in many catarrhines, which bear only one young normally, the presence of two placental disks is the rule—a primary disk to which the fetus attaches, and a secondary disk which develops subsequently.

Thus we interpret the present specimen as an instance of typical placentation in a monkey with dorsal and ventral placental disks in which the embryonic mass, however, has undergone twinning, producing single-ovum twins attached to the typically double placenta characteristic of most monkeys.

The condition of the membranes and placentae described is seen in figure 1. Two points are indicated (*anast.*) where anastomoses between the two circulations can be seen. Besides these features the two relatively large yolk sacs can be observed. These are well vascularized and are both in contact with the primary placenta. The cut margins of the amniotic sacs are visible. Because it has been necessary, for drawing the interior of the chorion, to open and spread the membranes and placentae apart, the size of one amniotic sac appears greater than that of the other. This has given a slight unavoidable distortion.

The placental disks are relatively thin, rather spread out, and moderately lobulated. The umbilical cords reveal, respectively, four blood vessels, two arteries and two veins.

MICROSCOPIC OBSERVATIONS

Search of the literature has failed to reveal any account of the microscopic anatomy of the marmoset's placenta. In view of the accepted primitive character of the Callitrichidae, compared to the other groups of monkeys, it has been of interest to contrast the microscopic structure of the placenta in the present instance with that of other platyrrhine and catarrhine monkeys.

Certain observations upon the microscopic structure of the placenta will be presented together with comparisons with other groups.

The placental labyrinth

The placental labyrinth is typically like the labyrinths of other platyrrhine monkeys from the middle period of gestation (Ateles, Alouatta; Wislocki, '29, '30). The labyrinth, instead of being of the villous type, characteristic of catarrhine monkeys and apes, is composed of trabeculae enclosing intertrabecular spaces as in Ateles and Alouatta (figs. 8 and 10). During the midgestational stage the trabeculae are relatively stout, containing much fetal mesoderm and clothed by a continuous layer of plasmoditrophoblast. There is in the present specimen less free syncytial trophoblast in the form of trabeculae than at a corresponding stage in Ateles and Alouatta. Presumably the proliferation of the plasmoditrophoblast is not quite as profuse as in Ateles and Alouatta, or the trophoblast has been at this stage more completely invaded by mesoderm. Cytotrophoblast is nowhere visible. The mesodermal cores of the stout fetal trabeculae are relatively cellular. As noted for other platyrrhines (Ateles; Wislocki, '30), there are numerous conspicuous fetal vessels in the trabeculae which are so large that they must be of the nature of sinusoids or venules (fig. 8). These vessels are

packed with large numbers of deeply stained nucleated blood cells with only a small proportion of erythrocytes. The significance of these vessels and their contained cells is not clear. The umbilical vessels and their branches seen on the surface of the placenta contain no such disproportion of nucleated cells.

Degenerative changes in the labyrinth are relatively scant. The formation of cell islands or of infarcts has not occurred. Deposition of fibrin is taking place in slight, scattered amounts in the closing plate and in a few of the major trabeculae. Stagnation of maternal blood, as takes place in a typically villous placenta, has very little opportunity to occur. The intertrabecular spaces are in many places so well filled with fresh maternal blood that the continuity of the maternal blood channels as tortuous, narrow passages between the trabeculae can be observed (fig. 10).

The lamina basalis

The structure of the lamina basalis is identical in many respects with that described in other platyrrhines (Wislocki, '29, '30). The pars glandularis is at this stage reduced to a narrow layer containing glandular clefts beneath the placenta (figs. 4 and 5). The glands are lined by rather large cuboidal cells which stain characteristically with eosin and exhibit small round nuclei profusely dotted with chromatin. Surrounding the glands, as well as the numerous maternal vessels approaching or leaving the labyrinth, is a stroma characterized by its cellularity (fig. 4). The cells of this stroma have indistinct boundaries and possess large, rather clear, oval or vesicular nuclei. Their nuclei stain pale blue, while the cytoplasm is a pale diffuse lavender in haematoxylin and eosin preparations. The glands terminate blindly in this cellular stroma. Not infrequently this is by proliferation of the glandular epithelium into cords or knots of cells which are walled off distinctly by the cells of the stroma (fig. 4).

A pars fibrosa is scarcely represented (fig. 5). Instead, the pars glandularis appears everywhere to come in contact

with the overlying layer, the choriobasalis. As a rule the demarcation of the aforementioned conspicuous stroma of the pars glandularis and of the elements of the choriobasalis is rather sharply defined, but in a number of places the two layers intermingle to some extent.

The choriobasalis is a very distinct narrow layer of tissue lying beneath the labyrinth (figs. 4 and 5). It is composed for the most part of strands or a network of plasmoditrophoblast possessing deeply staining nuclei. The trophoblast appears to be surrounded by or buried in a matrix which stains uniformly with eosin and is probably a form of fibrin.

To the choriobasalis the chorionic trabeculae are attached. The points of attachment are not nearly so numerous as in other platyrrhine monkeys. At the sites of attachment of the trabeculae the plasmoditrophoblast has undergone marked proliferation. The floor of the labyrinth between the bases of the attaching trabeculae is moderately infiltrated by fibrin and lacks for the most part a complete trophoblastic lining.

The placental border and the paraplacental endometrium

The placental borders terminate rather bluntly or taper to a wedge. The character of the border is rather simple. The closing plate and the choriobasalis meet and fuse to form a relatively thin chorionic membrane which has on its surface facing the uterus a layer of trophoblastic syncytium which may be one or several nuclear layers deep (fig. 11). The paraplacental endometrium is composed of an extension of the same cellular glandular layer that exists beneath the placenta. At the present stage, the glands in this layer do not appear to be either very extensive or particularly active (fig. 11). The majority of them do not seem to communicate directly with the uterine lumen, appearing instead to be sealed off by proliferation of the cells of the periglandular stroma or by detritus. In these respects the condition of the glandular layer resembles that described in catarrhine monkeys rather than that seen in other platyrrhines such as Ateles, Alouatta, and Cebus (Wislocki, '30). A series of placental

stages of marmosets will be required, however, to state definitely whether the glandular layer departs very radically in this regard from other platyrrhines.

The amniotic membrane, umbilical cord, and yolk sac

The amniotic membrane is clothed by a single layer of flattened cells. No epithelial thickenings or proliferations are encountered. The epithelium rests upon a narrow cellular mesodermal layer. Between the amniotic membrane and the chorionic membrane there extends an almost acellular gelatinous or mucoid substance in which the larger blood vessels pursue their way. The outermost layer of cells of the mesoderm of the amnion is in a state of proliferation. The cells of this sheet are enlarging, so that wandering cells are produced which migrate out irregularly into the acellular gelatinous material between amnion and chorion (fig. 6). Similarly, the blood vessels exhibit a conspicuous outer adventitial coat from which cells are proliferating and are wandering out into the surrounding gelatinous substance.

The umbilical cord contains in cross-section two umbilical veins and two umbilical arteries (fig. 9). These vessels are enclosed in a loose, relatively acellular connective tissue which is undoubtedly mucoid in nature. The epithelium investing the cord is for the most part flattened, although in places the cells appear to be cuboidal. No amniotic villi are present. No remnant of an allantois can be made out. A vitelline duct cannot be seen, but a relatively large vitelline artery and vein occur close together in one portion of the section (fig. 9). The walls of these vessels are degenerating, and stasis of blood within them appears to have taken place.

The yolk sacs are relatively large vesicles, as can be seen from examination of the gross specimen (fig. 1). Microscopically, their walls are seen to be thrown into a series of folds (fig. 7) which are clothed by indistinct, swollen cuboidal or low columnar cells. These cells contain within their cytoplasm minute droplets or granules. Within the stroma of the yolk vesicle there are numerous small blood vessels in which the blood has apparently ceased to flow.

DISCUSSION OF TWINNING IN MARMOSETS

It has been known for more than a century and a half that marmosets give birth as a rule to more than one young, generally twins. In spite of the numerous reports of twins in marmosets, I have sought in vain for a comprehensive description of the fetal membranes and placentae, and for a statement as to whether marmosets produce single- or double-ovum twins. Multiple pregnancy is occasionally cited as evidence that the Callitrichidae (Hapalidae) are primitive, a statement from which the inference must be drawn that the young are from multiple ova, because monozygotic twinning should be regarded as a specialization rather than a primitive condition. Moreover, the fact that several writers (Selenka, Weber) assume erroneously that platyrrhine monkeys have invariably a single placenta should necessitate their regarding the afterbirth of the marmoset, which consists of two placental masses, as an indication of multiovular twinning.

In the present instance it seems rather clearly established, after a careful examination of the membranes and placentae, that we have before us a case of single-ovum twinning. The evidence for this conclusion has already been given: the presence of two amnions with but one chorion; free anastomosis of the two circulations; and the existence of primary and secondary placental disks common to both fetuses, with the fetuses both attached to the primary disk. The present case of twinning in the marmoset can be best interpreted as an instance of single-ovum twinning in an animal form in which the blastocyst produces a bidiscoidal placenta.

The evidence that the blastocyst of the marmoset is normally bidiscoidal rests upon several arguments besides the observations on the present material. Fortunately, in the literature there is a brief description by Martin Saint-Ange ('44) of the fetal membranes of a marmoset which produced only one young. This observer says that there were two disks of equal size, one opposite the other, with but one young. Moreover, the umbilical cord and yolk sac attached to one disk, while the second disk was secondarily supplied

with blood vessels from the first. The present writer ('29, '30) has found a bidiscoidal placenta, as described in the marmoset by Saint-Ange, to be the usual condition for platyrrhine monkeys, with the sole exception of the howling monkey (Alouatta), which as a rule has a single discoidal placenta. Thus from my own observations, as well as that of Saint-Ange, the marmoset appears to be of the usual platyrrhine type in that it has a blastocyst which produces a bidiscoidal placenta. Consequently the two placental masses in the marmoset cannot in the presence of twins be regarded as derived from separate blastocysts.

Should the marmoset produce double-ovum twins, there would doubtlessly be four discoidal placental masses instead of two. This hypothetical condition has, to my knowledge, never been actually reported for the marmoset, but I have observed the condition in Lasiopyga, a catarrhine monkey, a specimen of which, bearing twins, is in my collection. Lasiopyga usually bears only one young with a bidiscoidal placenta. In my specimen bearing twins, however, there are two distinct chorions each possessing two discoidal placental areas, making four in all. This is clearly a case of double-ovum twinning, in contrast to the present case of single-ovum twinning in the marmoset.

In a catarrhine monkey (Cercocebus cynomolgus) Selenka ('92) has briefly described a case of monozygotic twinning identical, it appears, with the present marmoset specimen. Ordinarily the macaque produces one young with a bidiscoidal placenta. In Selenka's instance of twins he noted one chorion, two amnions, and two placental disks with both umbilical cords attached to the larger or primary placental disk. This, like the present specimen, is an instance of single-ovum twinning.

It is of interest to inquire whether single-ovum twinning as exemplified in this specimen is the rule for the marmoset. Were this the case, multiple pregnancy in marmosets would have to be looked upon as a form of specialization rather than a primitive condition associated with multiple ovulation.

Unfortunately, this is the only specimen at my disposal at present. The literature with which I am acquainted gives, however, one substantiating clue that single-ovum twinning may be the rule in marmosets.

Rudolphi (1828) describes briefly a marmoset placenta. He says that one chorion was present, with two amnions and two placental disks. From his brief description and figures one is led to infer that each fetus connected with a separate placental disk. This latter finding would be contrary to the observations upon the present specimen. However that may be, it appears likely that Rudolphi's specimen was also an instance of single-ovum twinning.

It would be of interest to learn whether marmoset twins are usually or invariably of the same sex. In the present instance the identical twins are both males. Of the nine pairs of marmoset twins mentioned in the literature which I have come across, in only one instance is the sex of the twins specifically stated. In this case (Virey, cited by Rudolphi) the twins were males.

In the fifteen cases of marmoset births known to me, eleven have been twins, three single, and one case a set of three. Besides the present case, in only two others, those of Rudolphi and Saint-Ange, have the membranes and placenta been briefly described. The case of three young is mentioned by Cuvier (cited by Rudolphi). This may have been an instance of combined single- and double-ovum twinning.

NOTES ON TWINNING IN MONKEYS IN GENERAL

Twinning in monkeys, other than the Callitrichidae, is not entirely unknown. In platyrrhine monkeys Schultz ('21) describes a pair of twin fetuses of the howling monkey (Aloutta seniculus). He says of these that from an examination of the fetal membranes it is evident that they are monozygotic or single-ovum twins.

In catarrhines twins have been more often observed. Selenka ('92) mentions a case of twins in one of the macaques, Cercocebus cynomolgus. He says that both umbilical cords

attached to the primary placental disk and that each fetus possessed its own amnion. This is evidently a case of monozygotic twinning identical with the condition reported here in the marmoset.

Fitzsimons ('19) records twinning in a baboon (Papio porcarius) and in a guenon monkey (Cerocopithecus pygerythrus), but does not report the nature of the fetal membranes.

To these statistics I can add the occurrence of twinning in a guenon monkey (Lasiopyga albigularis). The mother aborted two dead fetuses, one of which was deformed. The membranes consisted of two chorionic sacs each of which possessed two discoidal placental areas: evidently a case of double-ovum twinning. Normal for this species is to produce a single young with a typical double placenta. This case is the one referred to in the previous section. The material was obtained by Dr. Carl G. Hartman through the courtesy of the Chicago Zoölogical Park.

I am indebted to Doctor Hartman for another specimen which also has a bearing on this subject. This consists of the ovary of a macaque monkey in which two early corpora lutea are distinctly seen. Had these ova become fertilized, double-ovum twins would have resulted, with the formation of two double discoidal placental attachments.

In view of the relatively small number of monkey fetuses of which record has been made, these data suggest that twinning is not at all uncommon. In marmosets about 80 per cent of the pregnancies are multiple. In the other genera the incidence of twins may be about as great as in man.

CONCLUSION

The present examination of the fetal membranes and placenta is of value because it establishes from microscopic examination the close affinity of the placenta of Oedipomidas geoffroyi to those of other platyrrhines which are known. In regard to the structure of the placenta, Oedipomidas falls into a natural grouping with these other species. Twinning

in marmosets is a special feature of their reproduction. If, as the evidence adduced here seems to indicate, the twins are as a rule derived from a single ovum, the condition must be regarded as a specialization. Monozygotic twinning is fundamentally different from multiple ovulation, which is common in many mammals and is probably rightly deemed to be a primitive character.

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PLATES

PLATE 1

EXPLANATION OF FIGURE

1 Drawing of the placenta and fetal membranes of Oedipomidas geoffroyi. Notice the double discoidal placenta, the two umbilical cords, two yolk sacs, and two amniotic sacs. The umbilical cords both attach to the primary placenta. The blood vessels of the two fetuses share both the primary and the secondary placentae, numerous anastomoses occurring between the two circulations. Two such places of anastomosis are shown (*anast.*). This is an instance of monozygotic twinning in the presence of the typically double placenta characteristic of platyrrhine monkeys. × 2.

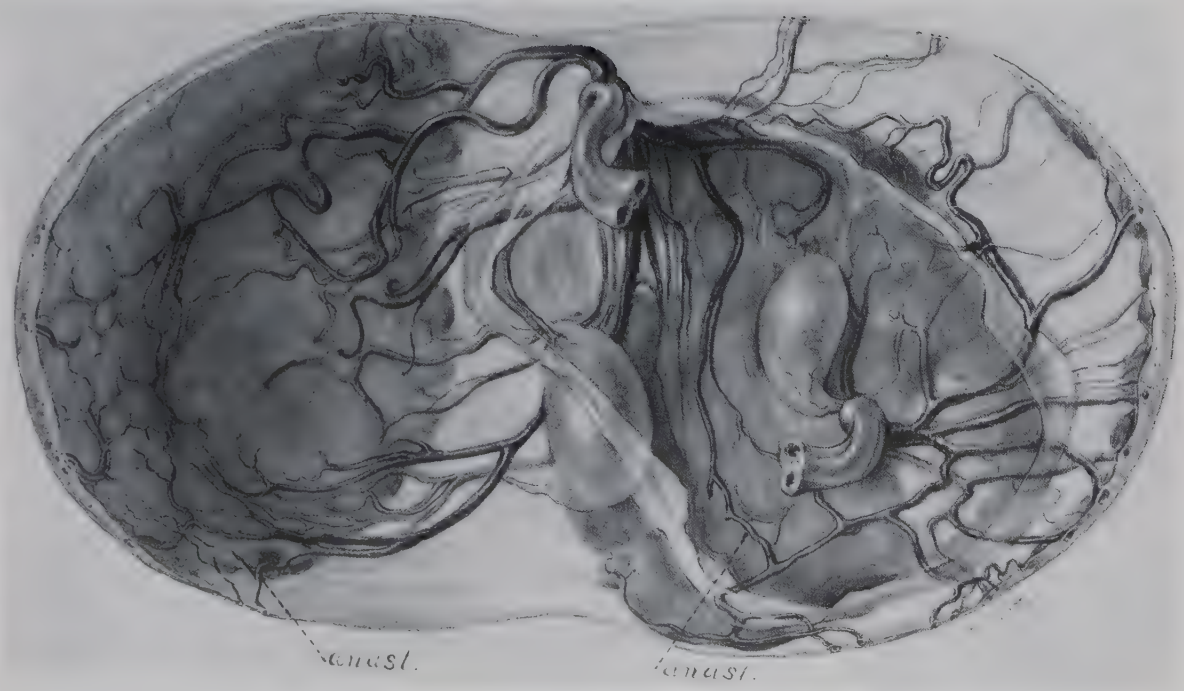
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PLATE 2

EXPLANATION OF FIGURES

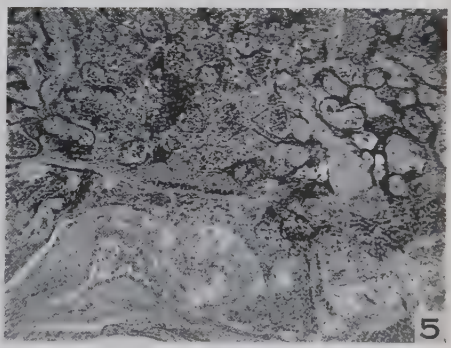
2 and 3 The twins, both males, removed from the uterus. × 1.

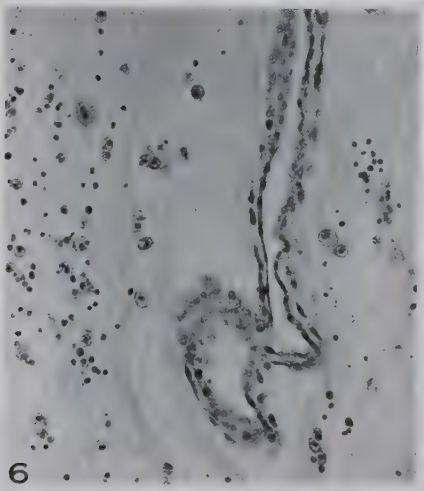
4 View of the basal part of the placental labyrinth, showing its attachment to the lamina basalis. Notice the pars glandularis of the lamina basalis containing several flattened, sealed-off uterine glands. Surrounding the glands is a rich cellular stroma. There is no distinct pars fibrosa of the lamina basalis. On the other hand, a distinct choriobasalis is seen beneath the labyrinth. × 160.

5 View of the lamina basalis similar to the preceding. × 160.

6 A portion of the amnion showing flattened amniotic epithelium and a narrow layer of associated mesoderm. From the latter, cells have proliferated and have wandered out into the acellular substance of the exocoelome. × 160.

7 View of the wall of the yolk sac with adjacent amnion. Between the two a field of unorganized material in which are numerous wandering cells. × 80.





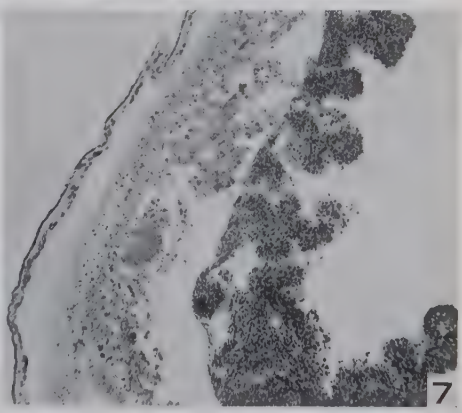


PLATE 3

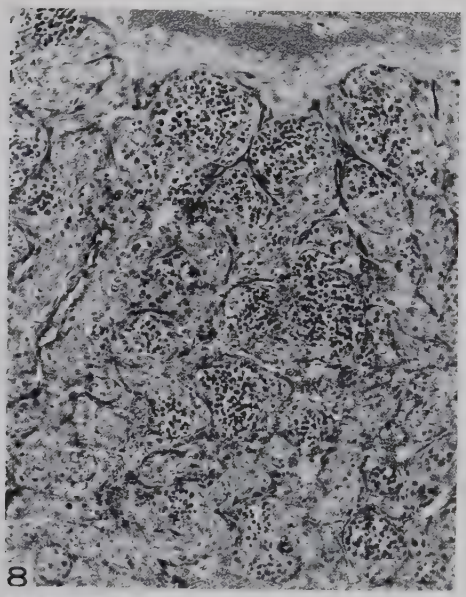
EXPLANATION OF FIGURES

8 View of the placental labyrinth showing the stout chorionic trabeculae closely packed together with scarcely any intertrabecular maternal blood spaces visible between them. The chorionic syncytium can be seen as dark lines on the circumference of the trabeculae. Within the trabeculae can be seen the wide venules packed with fetal leucocytes. × 180.

9 Cross-section of the umbilical cord of one of the fetuses, showing the two umbilical veins and the two very much smaller thick-walled umbilical arteries. In the neighborhood of the umbilical arteries are two dark spots, one smaller than the other, the degenerating vitelline artery and vein. × 28.

10 View of the entire width of the placental labyrinth in an area where the intertrabecular spaces are distended with maternal blood. × 80.

11 The paraplacental border showing the membranous chorion in loose apposition to the endometrium. The outer portion of the endometrium has been separated from the deeper layers of the uterus. The endometrium is cellular and partly degenerated. No surface epithelium is seen. The glands are cleft-like or slightly swollen and appear to be sealed off from the surface. × 80.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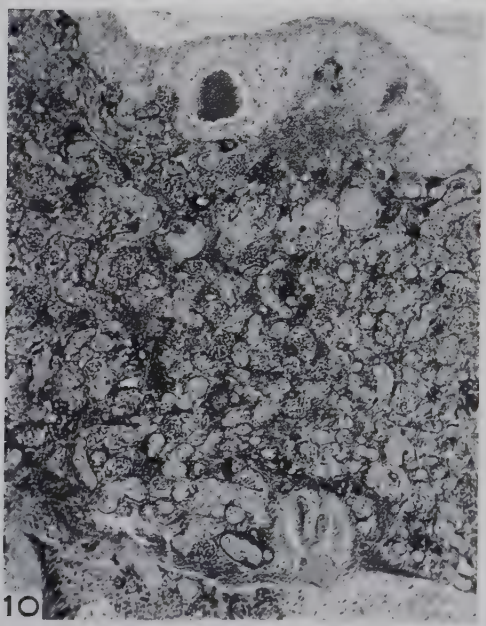
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THE MENSTRUAL CYCLE OF THE MALAYAN MONKEY, MACACA IRUS

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THREE FIGURES

Owing to the great interest now prevailing in the reproductive physiology of infrahuman primates and the considerable use which is now being made of various monkeys for experimental study of menstruation, it is important to gather as much information as possible about the menstrual cycles of individual species. Next to the rhesus monkey (Macaca mulatta), the commonest primates in zoos, at least in the United States, are the long-tailed Malayan macaques of the Macaca irus (= cynomolgus) group, of which many nearly related forms inhabit the Malayan mainland and archipelago, including the islands of Sumatra, Java, Borneo, and Banka. At the present time almost nothing is known of the reproductive physiology of the Malayan species, and therefore it seems worthwhile to report here observations which were made in my colony a few years ago.

The animals in question were part of a shipment of twelve young monkeys received on October 4, 1926, from a dealer in San Francisco. Although recognized at once to be different from the common rhesus macaque, they were not at first fully identifiable. They were characterized by having brownish-gray fur on the back, with a small patch of black or very dark brown hair on the top of the head between the eyes and ears. A vaguely defined streak of blackish color passed from this top-knot of black along the middle of the back almost to the tail. The hands and feet were dark gray,

almost black, and the tail was slightly longer than the body. Taken in connection with the general form, these characteristics were sufficient to identify the animals as belonging to the Macaca irus group. The skull and skin of one of the animals that died shortly after receipt were sent to Mr. Gerrit S. Miller, Jr., curator of mammals in the United States National Museum, who very kindly informed me that as far as he could determine from a young specimen, it was probably the mainland species M. irus. A race of this species from the coast of Siam shows, according to Mr. Miller, the same dark forehead.

I am further indebted to Mr. Miller for expert taxonomic advice upon which is based the following paragraph concerning identification of similar animals studied by workers in the physiology of reproduction.

Monkeys of the Macaca irus group have been called variously Cercocebus cynomolgos, Macacus (or Pithecus) cynomolgos, Cynomolgos fascicularis, and fifteen or sixteen other names. Some account of the synonymy and the distinguishing characteristics of these animals is given in Elliot's "Review of the Primates," volume 2, pages 229 to 234. By the first of these names Van Herwerden designates the monkeys which were studied by her in 1906. Joachimovits ('28) discusses the reproductive physiology of a species which he calls Pithecus fascicularis (mordax), but quotes as synonyms some of the other names listed above. Similar monkeys which form the basis of Spiegel's report ('30), to be quoted below, are called by the author Macaca irus mordax, but are identified with cynomolgos (Linn.), which as explained by Elliot (vol. 2, p. 231) really means the continental Malayan macaque M. irus. At any rate, the animals to be described in this paper and those studied by Van Herwerden, Joachimovits, and Spiegel may be considered for practical purposes as nearly related members of the Macaca irus group.

Joachimovits states, without citation of data, that animals observed in Java menstruated about every twenty-five to twenty-nine days, but showed wide variations up to thirty-

five days or more. The flow usually lasted three to five days. Zuckerman ('30) reports that one animal observed in the London Zoo showed fairly regular bleeding, four cycles averaging forty-one days. Other animals did not show menstruation, but the circumstances were such that the observations could not be fully relied on. Spiegel ('30) reports a total of forty-seven cycles observed in four animals. His results will be discussed below.

My animals proved to be rather susceptible to the autumn and winter climate, and a number of them died before we learned that they could not withstand outdoor life as well as M. rhesus. Four of them were, however, observed through twelve or more cycles each. It is believed that in each case the first onset of menstruation was observed, and that the animals may be estimated to have been about three or four years old during the period of observation.

To follow the menstrual cycles the animals were caught and examined daily except (usually) on Sundays, and vaginal smears were examined under the microscope. The criterion of the occurrence of menstruation was the same as that employed in my study of M. rhesus ('23), namely, any appearance of blood at the vaginal orifice lasting more than one day, providing that during part of the flow haemorrhage was macroscopically visible. Because of the usual omission of observation on Sundays, there is an occasional error of one day (plus or minus) in the records of cycle length and duration of flow. It is probable that all menstrual periods, as defined by the above criterion, were observed. The term menstrual interval as used here means the time from the day of onset of one period of menstrual haemorrhage until the day of onset of the next period. Protocols are given herewith.

The first fact which appears from these records is that the animals showed relative regularity of the cycle in the winter and a tendency to amenorrhea in the summer. This particularly noticeable in no. 30. The same phenomenon was hinted at with respect to M. rhesus in my report of 1923, page 81, and has been definitely confirmed by continued observations

Macaque no. 29. First observed menstruation, April 29, 1927

	Summer			*Autumn*			*Winter*			*Spring*		*Summer*			*Autumn*		
Intervals in days,	29	34	33	29	30	28	33	41	42	35	35	26	32	35	33	32	28	..
Duration of flow in days,	11	5	6	4	3	4	4	5	3	4	3	2	6	2	3	3	7	2

Macaque no. 30. First observed menstruation, May 16, 1927

	Summer	*Autumn*		*Winter*		*Spring*		*Summer*		*Winter*		*Spring*				
Intervals in days,	35	47	85	29	44	42	32	45	51	38	105	68	47	32	35	29	..
Duration of flow in days,	5	6	7	7	6	6	8	7	8	7	5	7	5	6	3	9	2

Macaque no. 33. First observed menstruation, September 30, 1927

	Autumn		*Winter*		*Spring*		*Summer*		*Autumn*		*Winter*		
Intervals in days,	26	39	35	42	40	35	52	31	111	67	24	27	..
Duration of flow in days,	5	8	11	12	9	8	6	5	13	9	6	8	9

Macaque no. 34. First observed menstruation, October 31, 1927

	Winter			*Spring*			*Summer*			*Autumn*			*Winter*			*Spring*		
Intervals in days,	32	31	49	30	30	34	42	35	33	27	29	25	32	28	33	31	63	..
Duration of flow in days,	4	4	6	4	4	2	2	3	3	4	5	4	5	4	2	2	3	2

on rhesus, made upon the animals of my Rochester colony. Dr. C. G. Hartman, in a paper to appear in the near future, has discussed this question in full as it concerns M. rhesus, and has reached the same conclusion from a large series of observations.

In analyzing data of this kind where there are seasonal or other breaks of regularity it is useless to consider averages. In determining, for example, the typical cycle length, the modal (i.e., the most frequent) cycle length is to be determined by plotting the frequency distribution of the intervals.

The graph (fig. 1) shows the frequency distribution of the intervals of these four animals. It will be observed that the

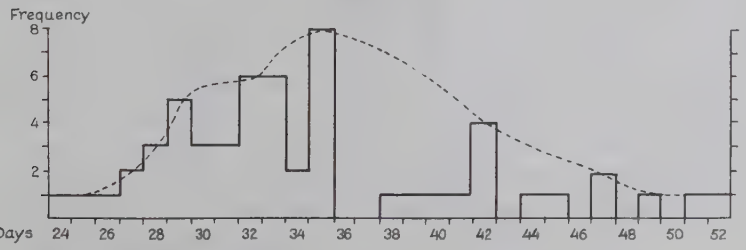

Fig. 1 Graph showing frequency distribution of menstrual intervals of Malayan macaques. Longer intervals, as follows, do not appear in this graph: one each of 63, 67, 68, 85, 87, 105, 111 days.

commonest frequency is thirty-five days, and that a curve drawn by connecting the tops of the individual columns (abscissae) shows a steep rise from twenty-four days to the mode and a more gradual descent. Curves of this type are characteristic of frequency distributions of menstrual cycles; ordinary symmetrical frequency curves of the Gaussian type do not occur because there are factors which skew the curve by limiting the occurrence of shorter cycles. Obviously there are great irregularities in this graph, due to the relatively small number of cases, so that the modal class (thirty-five days) falls next to a class (thirty-six days) having no members. For this reason the exact date at which the mode falls is not significant, and, in fact, inspection shows that if the large

class of day 35 were better distributed, the mode would probably be at an earlier day. The curve may be partially smoothed by plotting the data in groups of two or three days. When this is done, the mode falls on different days according to the way the groups are chosen, but in various experimental plots of this sort, the mode comes at between the thirty-first and the thirty-fifth day. The smoothest curve which may

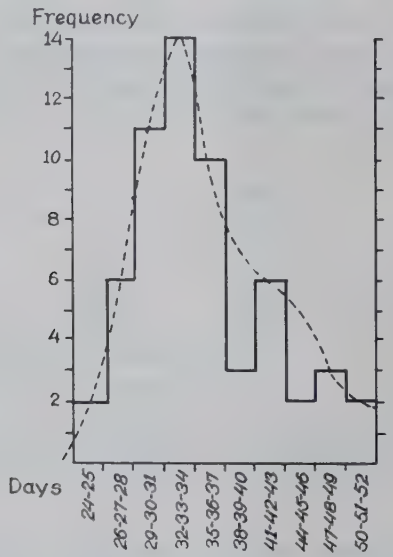

Fig. 2 Graph plotted from the same data as figure 1, but using groups of three days, in order to smooth out fortuitous irregularities.

justifiably be drawn from the data is that obtained by plotting in groups of three days as follows: days 23–24–25, 26–27–28, etc. The mode of this curve (fig. 2) falls at the group 32–33–34. It is obvious that the typical menstrual interval of these animals was four and one-half to five weeks.

This modal cycle length is a half week or more longer than the modal cycle length of M. rhesus as observed in the animals of my Baltimore colony reported in 1923, which was twenty-seven days. At is seemed not impossible that the difference

might be due to differences in climate and conditions under which the animals were kept rather than to any difference between the species, a further comparison was made by plotting the cycles of four rhesus monkeys which were housed in the Rochester colony at the same time as the Malayan macaques, and which were kept under identical conditions except that the rhesus monkeys were given more access to the open air in winter. The modal cycle length of these four rhesus monkeys again fell at about twenty-seven days, the frequency distribution curve (based on ninety-seven cycles) very closely resembling that determined from 125 cycles in 1923. This result strengthens the probability that the typical cycle length of M. irus is longer by at least three or four days than that of M. rhesus. Spiegel ('30) observed through a total of forty-seven cycles four Malayan macaques, all of which were considerably older than the four reported herewith, their ages being estimated at, respectively, six to eight, seven to ten, eight to ten, and twelve to fifteen years. It will be observed that the mode of Spiegel's graph falls on the thirtieth day. This is not far from the mode of the Malayan monkeys at Rochester.

In my four specimens of M. irus, the menstrual flow lasted from two to thirteen days. Periods of two, three, four, five, and six days were common, the commonest duration being four days. With respect to the duration of flow M. irus did not differ much from M. rhesus, in which the commonest duration seems to be four to six days, as reported in my paper of 1923 and confirmed by observations on the four Rochester rhesus monkeys used as a control on the four Malayan macaques. The duration of flow reported by Spiegel was shorter, the commonest duration being three days, but he makes it clear in his table that the figures given may, in many cases, be less than the true number by one, two, or three days.

During a considerable part of the time in which these four animals were under observation, a daily record was kept of the color of the so-called sexual skin, that is to say, the area of red edematous skin which is seen on the buttocks and

thighs and at the base of the tail. A color scale was prepared in ten gradations from the palest blue-red tint shown by these monkeys to the most intense red which the specialized skin may attain, and the individual monkeys were compared with the scale, the result being indicated by number from 1 to 10. It was found by this method that there is a strong tendency for the color to rise following menstruation, reaching its height at about the middle of the interval, and diminishing

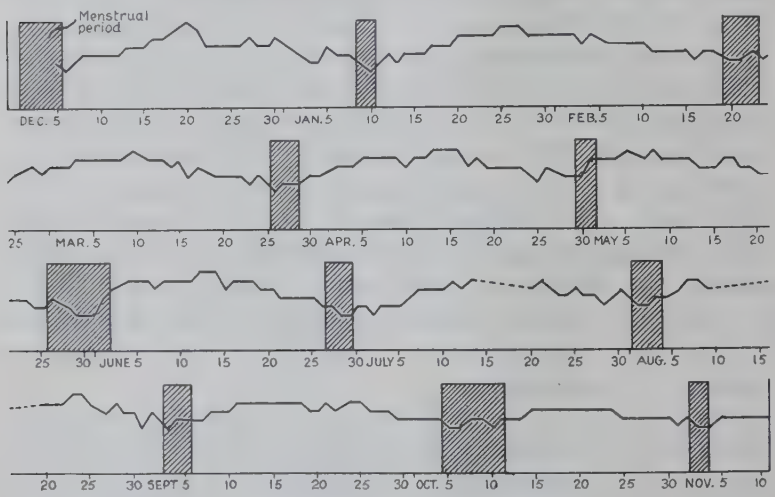

Fig. 3 Graph showing fluctuations of color of sexual skin of one Malayan macaque. The hatched bars indicate menstrual periods.

toward the onset of the next menstrual period. Such a periodic fluctuation of the color of the sexual skin is more or less well known to the keepers of zoos and has been discussed by Collings ('26) and by Hartman in a forthcoming paper. The same fluctuation appears in the rhesus monkeys of my colony. The appended graph shows the color of the sexual skin of the Malayan macaque no. 29 during her last ten cycles. The fluctuation is not always as regular as in this case, but in almost every instance it has been found, both in M. irus and M. rhesus, that the color is at its lowest ebb at the time

of onset of menstruation or during the flow. Since we know from the work of E. Allen and others that the color may be abolished by removal of both ovaries and restored by injection of oestrin (the ovarian follicular hormone), it is apparent that the color of the sexual skin is a rough indicator of the amount of oestrin available in the circulation of the animal. Such observations as these on the color of the sexual skin parallel the evidence recently brought out by Frank ('29) and by Siebke ('29) to the effect that menstruation in the human species is accompanied by a lower level of oestrin in the blood and in the urine. It should be said here that Doctor Hartman has found that in older rhesus monkeys the color change is much less marked than in the relatively younger specimens in my colony.

Vaginal smears or washings were made daily on the four Malayan macaques. The information gained from these smears was of the same type as that described in my paper of 1923 for rhesus. Traces of a cyclical variation were made out; the leucocytes tended to be low before the onset of menstruation, rising thereafter, while the epithelial cells tended to be more numerous during the latter half of the interval, up to the onset of menstruation, but these changes were not sufficiently distinct to be of use in predicting the events of the cycle.

CONCLUSIONS

1. Observations on four specimens of the Malayan macaque, Macaca irus, showed the typical menstrual cycle to have a length of about four and one-half weeks, with typical duration of flow from four to six days. The animals tended to become amenorrheic in the summer.

2. In other respects, the external events of the reproductive cycle closely resembled those of the related species, Macaca rhesus.

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NEW BOOKS AND PUBLICATIONS

NOTICES BY F. E. WELLS

A TEXTBOOK OF EMBRYOLOGY, by Mary T. Harman. 1932. Size, $5\frac{1}{2} \times 8$ inches. 476 pages. 284 illustrations. Index. Cloth. Price, $4.25. Lea & Febiger, Philadelphia.

With a simplicity and clearness well adapted to the student's needs, this text-book describes in detail the development of the individual from the egg until it is able to exist extraneously, and gives some attention to postembryonic development as well as to certain stages in the germ cells previous to fertilization. Although embryology is treated primarily as an anatomical subject, enough physiology has been included to show the relation between form and function and to emphasize the importance of structure in tissues, organs, and systems. Organogeny is outlined on the basis of a definite function for each germ layer. Embryology offers many questions not as yet thoroughly understood. The opinions of differing authors are included in this book for the purpose of stimulating thought, cultivating open-mindedness, and creating interest in this live and growing subject.

DER HERZALTERNANS, by Bruno Kisch. 1932. Size, $6\frac{1}{4} \times 9\frac{1}{4}$ inches. Pages xii + 214. Bibliography. Subject and author indices. 54 illustrations. Price, RM 20; bound, RM 21.50. Theodor Steinkopff, Dresden.

This book is one of a series edited by Bruno Kisch dealing with all phases of research on the circulation of the blood. The present volume is concerned with alternation of the heart's beat. It gives the methods—chemical, physical, and graphic—used in studying alternation, a description of its mechanism and nature, a comparison with other functions of the heart, and its effects on the circulation. A chapter of clinical importance is devoted to diagnosis, aetiology, therapy, and prognosis.

ZOÖLOGY, A Textbook for College and University Students, by F. E. Chidester. 1932. Size, $6\frac{1}{4} \times 9$ inches. Pages xii + 581. Index. Glossary. List of references. 268 illustrations. Buckram. Price, $3.75. D. Van Nostrand Company, Inc., New York.

In this survey of modern biology the author has included, along with the basic principles essential to a general culture course in zoölogy, enough physiology, ecology, and applied zoölogy to meet the needs of students entering medicine and agriculture. The introductory chapter gives definitions, descriptions, and classification. The succeeding chapters take up, in order, Protozoa, Porifera, Coelenterata, Platyhelminthes, Nemathelminthes, Annelida, Trochelminthes, Molluscoidea, Echinodermata, Mollusca, Arthoropoda, Chordata, Cyclostomata, Pisces, Amphibia, Reptilia, Aves, and mammals. The volume is completed with a discussion of the social life of animals, heredity, evolution, and eugenics. Important features of the book are the logical arrangement of facts about the animals within a group, a statement of the chief characteristics at the beginning of the discussion, and a summary of the economic importance at the end of each section.

REGENERATION UND TRANSPLANTATION, II. BAND: Transplantation, 2. Teil. By E. Korschelt. 1931. Size, $6\frac{1}{2} \times 10$ inches. 873 pages. 281 illustrations. Bibliography. Subject and author index. Price, 72 RM; bound, 75 RM. Gebrüder Borntraeger, Berlin.

With the second part of volume II the set is completed. The first volume, Regeneration, was issued in 1927, and both parts of the second volume, Transplantation, in 1931. They cover the subject exhaustively. Volume I discusses regeneration in plants and animals from the simplest one-celled organisms to the most complex, including man, comparing their regenerative capabilities, commenting on special phenomena and underlying principles. The second volume describes the methods and technique employed in experimental transplantation, and the results of research carried out on many kinds of specimens. Part II, just issued, tells of surgical transplantation of skin, sense organs, nervous system, and other organs and tissues. It contains the summary and conclusions. The bibliographic references are grouped by subject, following the arrangement of the text.

LA THÉORIE PHYSICO-CHIMIQUE DE LA SEXUALITÉ, by Ph. Joyet-Lavergne. 1932. Size, $4\frac{1}{2} \times 7$ inches. 102 pages. Illustrated with 5 text figures. Price, 10 francs. Gaston Doin et Cie, Paris.

Technical details and bibliography have been omitted intentionally to make this a book for the general reader rather than for the specialist. It explains the physicochemical conception of sex and its bearing on sex determination, intersexuality, reversal of sex, and other aspects of the subject.

NEW BOOKS AND PUBLICATIONS

NOTICES BY F. E. WELLS

THE MICROSCOPE, by Simon Henry Gage. Ultraviolet (15th) Edition. 1932. Size, $6\frac{1}{4} \times 9\frac{1}{4}$ inches. Pages, viii + 586. 291 illustrations. Index. Bibliography. Table of Measurements and Natural Sines. List of References for Collateral Reading at the end of each chapter. Cloth. Price, $4.00. The Comstock Publishing Company, Ithaca.

This new edition of Professor Gage's book is called the ultraviolet edition because an entirely new chapter on the ultraviolet microscope and its use has been added. It also emphasizes the physical analysis of structure by means of the dark-field, the ultraviolet, and the polarizing microscopes. The beginning student working for the first time with the microscope will find in this book just such information as he needs—accurate definitions, descriptions and pictures of the apparatus and all its parts, and instructions on their use. He will find the guidance by which he can gain intelligent understanding of the microscope, its limitations, and its possibilities. He will learn the underlying principles and reasons for the methods used in preparing objects for study and the interpretation of their appearances when seen under the microscope. The author writes with the authority of one who knows intimately from his own experience every step in the use of the microscope. He points out the difficulties that will be encountered, tells how they may be avoided or overcome, and demonstrates with numerous practical exercises the theoretical part. The experienced research worker using this as a reference book will delight to find described in one volume the best of modern instruments and techniques. It is intensely practical—besides describing new apparatus it tells, whenever possible, how similar good results may be obtained by the use of such material as is likely to be on hand in the laboratory. It also contains a full discussion of drawing with the microscope and with the projection apparatus; fixing and preservation of tissues, organs and organisms; sectioning; photography; mounting, labeling, and storing microscopical preparations. A brief history of lenses and microscopes concludes the book.

LIFE HISTORIES OF THE FROGS OF OKEFINOKEE SWAMP, GEORGIA. North American Salientia (Anura) No. 2, by Albert Hazen Wright. 1932. Size, $7 \times 10\frac{1}{2}$ inches. Pages, xv + 497. Illustrated with 45 plates made up of 387 photographs from life. Index. Bibliography. Cloth. Price, $8.00. The Macmillan Company, New York.

A comprehensive study of the frogs of the Okefinokee Swamp, located in the State of Georgia, and belonging to the Sabalian and Lower Austral life zones. This report is a result of expeditions sent out by Cornell University whose object was "to study and put on record something of the biological conditions in this fresh-water swamp, which still presents in a large measure primitive and interesting conditions of environment, before they should become forever changed by the now rapidly penetrating lumbermen." The book is based on material collected

by the author and his parties in the swamp, and on the collection belonging to the United States National Museum. A general discussion of the Okefinokee salientia compares them with salientia from other parts of the country and gives tables, keys, and general summaries. Life histories of twenty-two species are fully described, each with abundant information on scientific name, common names, range, general appearance, measurements, habitat, first appearance, general habits, voice, mating, ovulation, egg-laying, eggs, hatching period, tadpoles, larval period, transformation, growth, food, autumnal disappearance, and affinities. A list of bibliographic references follows each life history.

THE MACHINES WE ARE, The Principles of Living Phenomena, by Robert T. Hance. 1932. Size, 6 × 9 inches. 398 pages. Index. List of suggested readings. Price, $3.00. Thomas Y. Crowell Company, New York.

Most of the essays in this interesting book were presented as a series of radio talks and were so enthusiastically received that they were preserved in book form for supplementary reading in a general course in zoölogy. They are written in a light easy style which amuses and stimulates the reader while giving him some knowledge of biological principles. The author's foreword outlines the contents: "We shall begin with heredity, since the majority of people are more or less familiar with its results in the human race and are usually interested in the subject although frequently wrong in their observation. Next in line will be a discussion of reproduction, for naturally there can be no heredity until reproduction takes place. Reproduction cannot be consummated without the cooperation of all the body tissues whose combined activities result in the phenomenon which we call life. The organism could not long exist without some means of keeping in touch with its surroundings; and so nervous reactions must be considered. Between the discussions of these three great biological principles of body-building, nervous response, and reproduction have been inserted essays of such subjects of general interest as immunity to disease and control of abnormally functioning organs. This has been done both to give an idea of the variety of things a living animal must perform in order to keep on living and to raise the question as to the eventual extent of man's control over vital phenomena. And so we come to the place where the relationship of all living things calls for discussion, and this leads to the final essays in which are outlined the contributions that biology has made and can still make to human comfort and happiness."

April No 3

NEW BOOKS AND PUBLICATIONS

NOTICES BY F. E. WELLS

SPEECH PATHOLOGY, by Lee Edward Travis. 1931. Size, 5¾ × 8¾ inches. Pages xxiv + 331. Index. Glossary. Illustrated. Cloth. Price, $4.00. D. Appleton & Co., New York.

This book gives a concept of the human organism as a dynamic whole, functioning as a unit under graded centers of physiological dominance, the term physiological dominance being used to mean the control of less active parts by more active parts. The brain centers, and the cerebral cortex in particular, are cited as being the organs of highest dominance. Speech, a relatively recent biological development, uses structures which ordinarily subserve other and different functions. In normal conditions the entire peripheral speech mechanism functions as a unit under one dominant gradient of control in the cerebral hemispheres. Constitutionally, one or the other hemisphere is dominant, as is manifested in right- or left-handedness. Factors interfering with this one-sided dominance disturb the development of perfect speech. Disabilities of speech are classified as disorders of verbal rhythm (stuttering), disorders of articulation and phonation, and disorders of symbolic formulation and expression. The general causes of defective speech include heredity, pathological foetal positions, birth injuries, training which conflicts with innate handedness, physical injuries and diseases, and general mental deficiency. Diseases of speech are studied as to their neurological concept, signs, causes, nature, and management. The appendix gives fifty-three pages of stimulus syllables, words, and sentences for speech training.

FUNDAMENTALS OF INSECT LIFE, by C. L. Metcalf and W. P. Flint. 1932. Size, 6 × 9 inches. 581 pages. 315 illustrations. Index. Bibliography. Buckram. Price, $5.00. McGraw-Hill Book Company, New York.

Emphasis on the biological rather than on the agricultural aspects of entomology make this an excellent text-book. The authors have brought together and simplified a wealth of information and fascinating facts stimulating the interest of the student or general reader and giving him some knowledge of insects and their activities, both harmful and helpful. As enemies to man, insects injure all kinds of growing crops and valuable plants; they attack and annoy man and other animals, and they destroy and contaminate stored products and possessions. Beneficial activities of insects include the production and collection of useful products, such as silk and honey, pollenization of blossoms, improving of soil fertility, etc. Anatomy and physiology, development and metamorphosis, and the place of insects in the animal kingdom are capably treated. A classification arranges them by orders and families. Chapters of unusual interest tell about insect behavior and the biology and ecology of insects in relation to their living environment and their physicochemical environment.

CYTOLOGY AND CELLULAR PATHOLOGY OF THE NERVOUS SYSTEM, edited by Wilder Penfield. Three volumes. 1932. Size, 6¼ × 9¼ inches. 1280 pages. 886 illustrations (15 in color). Index. List of bibliographic references following each section. Cloth. Price, $30.00. Paul B. Hoeber, Inc., New York.

A remarkable set of volumes which might be described as a complete library on the microscopic structure of the nervous system. The editor has done a great work in bringing together chapters by twenty-six celebrated authors—an accomplishment of inestimable value to the clinician, the neurologist, the anatomist, the pathologist, the histologist, and the surgeon. It makes available for the first time in English the work of many of the contributors. The general plan of construction is the same as that of 'Special Cytology,' edited by Dr. E. V. Cowdry. The treatment of each subject is largely determined by the interests of the section author, and he alone is responsible for the opinions expressed. In the preface Doctor Penfield outlines the contents of the volumes. "Volumes I and II take up the cytology and the cellular pathology of the nervous system in health and disease, without treating the disease entities under separate headings. In them the anatomy and pathology of each cell group are described in turn, thus presenting information which is prerequisite to the understanding of more specialized work. The third volume contains studies of certain pathological entities for which the more fundamental contributions in volumes I and II are a preparation. The descriptions which are scattered through both volumes are gathered together and made available to pathologists in the general index." The articles included in the three volumes are as follows:

The Neurone. General Character, by E. V. Cowdry. Principles of Development on the Nervous System (Neurobiotaxis), by C. U. A. Kappers. Sensory Ganglia of the Cranial and Spinal Nerves. Normal and Pathological, by F. de Castro. Histopathology of Nerve Cells, by M. Bielschowsky. Sheaths of the Peripheral Nerves. Nerve Degeneration and Regeneration, by J. Nageotte. Nerve Endings. Motor and Sensory, by J. Boeke. Sympathetic Ganglia. Normal and Pathological, by F. de Castro. Nerves of the Blood Vessels, Heart, Meninges, Digestive Tract, and Urinary Bladder, by P. Stöhr, Jr. Neuroglia. Normal and Pathological, by W. Penfield. Microglia, by P. Del Rio-Hortega. Choroid Plexus and Ependyma, by E. Agduhr. Cerebrospinal Blood Vessels, by Stanley Cobb. The Meninges, by Lewis H. Weed. Pineal Gland, by P. Del Rio-Hortega. Hypophysis Cerebri, by Paul C. Bucy. Retina, Chorioid, and Sclera, by L. B. Arey. Optic Nerve and Papilla, by William Cone and J. A. MacMillan. Primary Tumors of the Brain, by Percival Bailey. Tumors of the Sheaths of the Nervous System, by Wilder Penfield. Primary Tumors of the Spinal Cord and Intradural Filum Terminale, by J. W. Kernohan. Tumors of the Optic Nerve, by F. H. Verhoeff. Tumors of the Retina, by Roy Grinker. Tumors of the Chorioid and Allied Tumors, by Jonas S. Friedenwald. Neuroblastic Tumors of the Sympathetic Nervous System, by M. Bielschowsky. Neural Proliferations in the Vermiform Appendix, by Pierre Masson. Tumors of the Hypophysis Cerebri, by Percival Bailey. Malformation in the Central Nervous System, by J. H. Globus. Hydrocephalus and the Atrophy of Cerebral Compression, by W. Penfield and A. R. Elvidge. Inflammatory Cells in the Central Nervous System, by J. C. Greenfield. Cells in the Cerebrospinal Fluid, by W. Boyd.

May No. 4.

NEW BOOKS AND PUBLICATIONS

NOTICES BY F. E. WELLS

REFLEX ACTIVITY OF THE SPINAL CORD, by R. S. Creed, D. Denny-Brown, J. C. Eccles, E. G. T. Liddell, and C. S. Sherrington. 1932. Size, $5\frac{1}{2} \times 8\frac{3}{4}$ inches. Pages viii + 183. Index. 71 illustrations. List of references. Cloth. Price, $3.25. Oxford University Press, New York.

A concise account of elementary features of reflex mechanism as illustrated particularly by the mammalian spinal cord. Actions involving nerve elements higher than the quadrigeminal region are omitted and only reflexes in the strict meaning of the term and obtainable from a mammalian preparation are considered: The reflex arc, the spinal gray matter, the flexor reflex, the stretch reflex, reflexes in extensor muscles other than postural reflexes, central inhibition, and lower reflex coordination. The discussion is concerned with problems of physiological production and execution rather than those of biological significance. The individual motor unit is stressed as a basal element of the reflex response.

GROWTH AND DEVELOPMENT OF THE MUTTON QUALITIES OF THE SHEEP. A Survey of the Problems Involved in Meat Production, by John Hammond, with a section in conjunction with A. B. Appleton. 1932. Size, $6\frac{1}{2} \times 10$ inches. Pages, xxxvi + 597. Illustrated with drawings, diagrams, charts, tables, and photographs. Subject and author indices. Bibliography. Price, 42 shillings. Oliver and Boyd, Edinburgh.

The scientific principles involved in the production of meat are considered in this general survey from the physiological, anatomical, and practical points of view. The study approaches the problem by observations on the product—meat—and works backward to find out the conditions and factors which affect its formation. It investigates the factors which influence rate of growth in live weight and determines the composition of the growth made at different periods in terms of body organs and tissues. The main divisions of the book are as follows: The Rate of Growth in Live Weight in a Flock of Suffolk Sheep. The Carcass Percentage and the Relative Development of the Different Organs in the Body. Variations in the Rate of Development of Different Parts of the Skeleton (Body Conformation). Variations in the Proportions of Muscle Fat and Bone in the Carcass. Study of the Leg of Mutton.